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The Cell Migration in the Adrenal Cortex of Rats Studied with Tritiated Thymidine

By

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Abstract

DIDERHOLM, H. and B. HELLMAN. *The cell migration in the adrenal cortex of rats studied with tritiated thymidine.* Acta physiol. scand. 1960. *50.* 197—202. — Changes in position of the adrenal cortical cells of the rat were studied by autoradiography using tritiated thymidine. Four hours after the thymidine injection, the labelled cells were localised to the capsule, zona glomerulosa and the outer parts of the zona fasciculata. After one month, many strongly labelled cells were found as far down as the transition region of the zona fasciculata and the zona reticularis, while after two months, they were found within the zona reticularis. In the discussion of the results, it was taken into account that each zone of the adrenal cortex is considered to produce its own kinds of hormones.

On the basis of his observations of mitotic figures in the outer parts of the adrenal cortex and an abundance of degenerative cells bordering on the medulla, GOTTSCHAU as early as 1883, advanced the theory that a continuous renewal of cortical cells takes place in the outer part and that, with increasing age, they are displaced centripetally. However, although many authors still adhere to a more or less modified "migration hypothesis", recently this has been superseded to a large extent by the transformation theory of TONUTTI (1953). The strongest argument against a continuous inward "migration" of adrenal cortical cells and their final destruction in the zona reticularis, is put forward by the authors who maintain that every zone of the cortex has its own specific function (DEANE, SHAW and GREEP 1948, GREEP and DEANE 1949, HARTROFT and EISENSTEIN 1957). It is therefore difficult to accept *a priori* the idea that a cell "migrating" through the cortex produces mineralocorticoids in the zona glomerulosa, and that later in the zona fasciculata it is

responsible for the secretion of glucocorticoids. A detailed presentation of the different theories concerning the life cycle of the cells of the adrenal cortex has been given in an excellent review by BACHMANN (1954).

When considering the controversial ideas which are advanced to explain possible changes in position of the cells of the adrenal cortex, it must be remembered that adequate methods of studying this problem have not previously been available. SALMON and ZWEMER (1941), when injecting trypan blue into rats, noticed that after one day, particles of the dye were found in the capsular cells, and after about a month, in cells as far down as the zona reticularis. They regarded this as proof that centripetal displacement really existed. However, according to later authors, these conclusions could not be drawn from the latter experiment, since these observations can be quite simply explained by a diffusion of dye in combination with a varying affinity of the cortical zones to trypan blue (CALMA and FOSTER 1943, MCPHAIL 1944, BAXTER 1946, WALLRAFF 1949).

A convenient method of dealing with the present problem is now available, as a result of the introduction of the precursor of DNA, thymidine, which after being labelled with tritium, can be used for the autoradiographic recording of the formation and "migration" of cells in different organs. By studying the distribution of radioactive cells four hours after an injection of tritiated thymidine, it has been possible to show that cell proliferation in the adrenal cortex of the rat is localised to its outer areas (DIDERHOLM and HELLMAN 1960). Except in connection with cell division, the stability of DNA is such that the radioactivity in the cells does not change for a long time (LEBLOND, MESSIER and KOPRIWA 1959, MACDONALD and MALLORY 1959). Because of this, it is now possible for the experiments to be extended to include the fate of adrenal cortical cells labelled with tritiated thymidine, with particular reference as to whether or not they show any centripetal "migration".

Material and Methods

Fourteen male rats 21 days old were used for the investigation. Thymidine-H^3 with a specific activity of 2.7 Curies/millimole (Schwartz Laboratories, Mt. Vernon, New York), was injected i. p. in the dose of 1 μC/g body weight. The animals were killed by means of ether inhalation at the following times after injection:

A) Four hours (7 rats). B) One month (4 rats). C) Two months (3 rats).

The adrenals were removed immediately and fixed in 10 % formalin. After the usual dehydration and clearing, the specimens were embedded in paraffin and then cut into sections 5 μ thick.

The stripping procedure was in the main carried out according to the methods of DONIACH and PELC (1950), using Kodak autoradiographic stripping plates (AR 10). The sections were stained with haematoxylin.

Results

The regional distribution of the radioactive cells in the adrenal cortex was greatly influenced by the length of time which had elapsed between the injec-

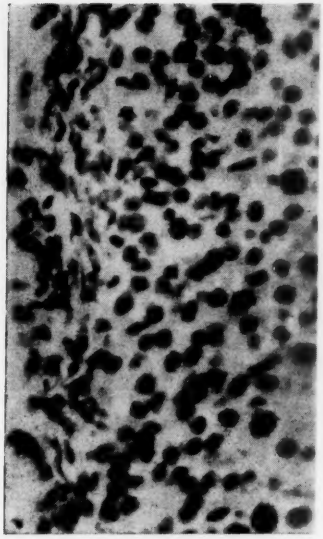

Fig. 1.

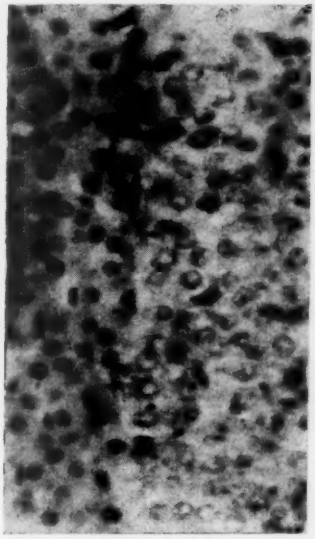

Fig. 2.

Fig. 1. The capsule, the zona glomerulosa and the outer areas of the zona fasciculata from the adrenal cortex of a rat, which was killed four hours after the injection with tritiated thymidine. About 10 labelled cell nuclei can be seen, mainly in the outer areas of the zona fasciculata. Magnification × 700.

Fig. 2. The border between the adrenal cortex (on the left) and the medulla (on the right) in the same rat as in Fig. 1. Only one weakly labelled cell can be seen in the medulla. Magnification × 700.

tion of tritiated thymidine and the deaths of the animals. In those rats which were killed 4 hours after injection, labelled cells were found mainly in the capsule of the organ, the zona glomerulosa, and the outer areas of the zona fasciculata (Fig. 1). The highest concentration of labelled nuclei, corresponding to 10—15 % of the cell total, was found in the transition region of the zona fasciculata and zona glomerulosa. Towards the deeper layers of the adrenal cortex, the frequency of radioactive nuclei rapidly decreased, so that the inner half was almost free of labelled cell nuclei (Fig. 2). On the other hand, a month later, the majority of labelled cells were found in the latter area, and then mainly in the inner third of the zona fasciculata. In this area, the frequency of labelled cells amounted to 3—6 %. The nuclei here showed every degree of blackening, but a large number of them appeared to be very deeply blackened. A month after the thymidine injection the deepest lying labelled, cortical cells had reached the outer areas of the zona reticularis. In the capsule, the zona glomerulosa and the outer areas of the fasciculata, the frequency of labelled nuclei had now diminished to only 1—2 % of the total, and these were only slightly radioactive.

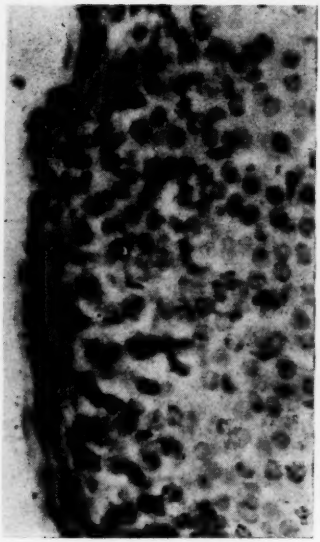

Fig. 3.

Fig. 4.

Fig. 3. The capsule, the zona glomerulosa and the outer areas of the zona fasciculata from the adrenal cortex of a rat, which was killed two months after the injection with tritiated thymidine. Only weakly labelled cells are seen. Magnification × 700.
Fig. 4. The border between the adrenal cortex (on the left) and the medulla (on the right) in the same rat as in Fig. 3. Strongly labelled cells are seen in the zona reticularis near the border between cortex and medulla. Magnification × 700.

In those rats which were killed two months after the injection, the proportion of radioactive cells in the latter area had diminished further, so that the frequency of cells, all only slightly radioactive, even in the transition region of the zona glomerulosa and the zona fasciculata, never exceeded 0.5 % (Fig. 3). By contrast with the animals which had been killed one month earlier, the majority of the labelled cortical cells showed a continued "migration" towards the medulla and were now mainly found in the innermost areas of the zona fasciculata and the zona reticularis (Fig. 4). The labelled cell nuclei in question, amounting to 1—4 % of the total, showed considerable variation in the degree of blackness. In the last mentioned areas, many strikingly labelled cell nuclei were found, as well as others slightly radioactive.

In comparison with the cortical cells, the radioactive medullary cells showed no marked difference in localisation, degree of blackening, or frequency, when the interval between the thymidine injection and death was extended to one or two months. After two months the frequency of labelled medullary cells, initially exceeding 1 %, tended to be less, at the same time as the initially weak blackening also diminished.

Discussion

The time, during which the injected thymidine is available for DNA synthesis, is so short (HUGHES *et al.* 1958), that the labelled cells which were observed after one and two months respectively are considered to be either cells which were already labelled after four hours, or cells derived from these. This interpretation is based on the report that nucleic acid catabolites are not reutilized for nucleotid synthesis to any great extent (DANCIS and BALIS 1954). As far as the adrenal cortex is concerned, it seems justified in interpreting the new formation of cells as arising through mitotic division (BACHMANN 1954). Thus the fact that a number of cells were still very strongly labelled one and two months after thymidine injections depends on these cells having not divided or having divided only once.

A "migration" of cells from the outer areas of the adrenal cortex is likely, since it had already appeared from the experiment with tritiated thymidine that at the time the experiment was performed, new formation of cells was almost exclusively localised to that part of the cortex (DIDERHOLM and HELLMAN 1960). However, direct studies of the changes in position of the cells labelled with tritiated thymidine were justified since the need for new cells in the zona reticularis might also be satisfied by a periodic return of mitotic activity. Autoradiograms showed that the longer the time which had elapsed following the thymidine injection, the further down in the adrenal cortex strongly labelled cells were to be found. On the other hand, those cells which were still in the outer part of the adrenal cortex after one and two months were all only slightly radioactive. It is therefore justifiable to distinguish between two different types of cells; one more active mitotically and remaining in the outer areas of the adrenal cortex, and the other, with less tendency to mitosis, and in comparison with the cortical zones continually changing its position towards the medulla. It should be remembered that during the experimental period, the adrenal glands had grown and the volume ratio of the different zones had changed. For this reason it cannot be determined for the time being, to what extent the striking change in position of the labelled cells in relation to the three cortical zones depends on a real displacement or whether it merely appears to be so because of differences in the reconstruction and extension of the zones.

If it is accepted that the zones have different functions, it is somewhat surprising to observe that a cell, which at one time is found in a certain zone, is later to be found in a deeper zone. Thus in the rat, there is experimental support for the idea that the zona glomerulosa secretes mineralocorticoids, while the zona fasciculata forms 11-oxysteroids (HARTROFT and EISENSTEIN 1957, etc). While the zona reticularis has often been associated with the production of androgens, no special zone has been held responsible for their origin in the rat (PASCHKIS, RAKOFF and CANTAROW 1958). If the concept is held that the three different zones have separate functions, *i. e.* that the cortex is functionally

divided into three, then one and the same cell during its "migration" towards the medulla does not produce the same hormone. On the other hand, the views of different functions in the zona glomerulosa and zona fasciculata only, *i. e.* functional division of the adrenal cortex into two, does not necessarily imply that the cortical cells adjust their production to that of a new hormone with increasing age. The original glomerulosa cells never actually need to leave this zone, but it is possible that the radioactive cells which were observed in the lower areas of the zona fasciculata and zona reticularis only came from those cells, originating in the outer third of the zona fasciculata.

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The Activity of Muscle Receptors in the Kitten

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Abstract

SKOGLUND, S. *The activity of muscle receptors in the kitten.* Acta physiol. scand. 1960. *50.* 203—221. — In the present paper which is a continuation of earlier publications on the postnatal development of postural reflexes, the activity of muscle receptors is studied in kittens from birth to 45 days of age. It is found that the discharge of receptors in the gastrocnemius muscle in response to stretch is phasic in character in the newborn and young kitten, while tonic responses are obtained from more proximal muscles around the hip and spine. Even the selective strongly tonic effect of succinylcholine iodide on muscle spindles is missing. Similarly, at that time the response of the Golgi tendon organs is phasic. With increasing age of the kitten more sustained discharges appear though in the beginning the rate of firing is low in the gastrocnemius muscle. Evidence is presented to show that the appearance of tonic discharges is in some way related to parallel changes in the electrical properties of the nerve fibres and the mechanical properties of the muscle, but further work is needed to elucidate the full significance of this finding. It is also shown that gamma control of muscle spindles in the gastrocnemius can be demonstrated 17—20 days after birth. Evidence is further presented to the effect that gamma control of muscle spindles in proximal muscles appears earlier, and that the gamma control of spindles in distal muscles is preceded by development of an alpha mechanism of spindle control. The obvious significance of these findings for the postnatal appearance of tonic stretch reflexes and decerebrate rigidity is discussed.

In recent publications (SKOGLUND 1960 a) it was demonstrated that the tonic stretch reflex is lacking in distal limb muscles of newborn kittens. Later evidence (SKOGLUND 1960 b) showed that the afferents of these muscles already at birth have established monosynaptic connections with the ventral horn cells. Long reflex times were, however, found which could be shown to depend mainly on slow peripheral conduction rates. The combination of missing tonic stretch reflexes, presence of monosynaptic connections and good reflexes from other sources — the crossed extensor was for instance present (SKOGLUND 1960 a) — focused attention on the nature of the deficiency in the reflex response to the afferent inflow from muscle receptors. The first question asked was whether muscle spindles function in the normal way in muscles lacking tonic stretch reflexes. Would for instance the spindles themselves be immature enough or would gamma drive be deficient? From the work of ELDRED *et al.* (1953) and MATTHEWS and RUSHWORTH (1957) we know that gammasupport is necessary for tonic stretch reflexes. The gradual appearance of these reflexes points to a delayed postnatal development of the afferent link including delayed development of gamma control. This will be studied below. In a later paper (SKOGLUND 1960 c, d) will be shown that postnatal maturation by no means is confined to the muscle spindles and their gamma control but that it also includes central connections and the properties of the afferent terminals.

Methods

Kittens from 35 different litters have been used ranging in age from 1—45 days. The animals were anesthetized with Nembutal 20—35 mg/kg body weight, given intraperitoneally. In all some 80 muscle spindles and 20 tendon organs have been studied.

Laminectomy was performed in the lumbar region of the cord and all dorsal and ventral roots from L II downwards were severed at their exit from the cord. Usually the ventral roots L 7 and S I were used for stimulation to set up a contraction in the gastrocnemius muscle which was dissected free. Its tendon was connected to the steel tongue of an isometric strain gauge myograph. The limb was fixed at knee and hip with drills in the usual manner. The discharge from the muscle receptors was generally recorded from isolated filaments of the dorsal roots L 7 and S I but sometimes also from dissected filaments of the muscle nerve. It was attempted to cut all nerves to the limb other than those running to the gastrocnemius muscle under investigation. A pair of electrodes was often placed in the popliteal fossa for stimulation of the muscle nerves and to measure conduction times according to the method earlier described (SKOGLUND 1960 b). All dissected nerves and roots were covered by paraffin pools at 37° C.

Intra-arterial injections of succinylcholine iodide (Celocurin, Vitrum) was given at the aortic bifurcation through a cannula introduced in the contralateral femoral artery.

The action potentials recorded were fed to a push-pull amplifier through a cathode follower input and displayed on one of the beams of a double-beam cathode ray oscilloscope whose second beam recorded either muscle tension or time as, given by a sine wave generator. The response of the sense organs was also monitored by a loudspeaker. The peripheral nerves and roots were stimulated by square wave shocks with a duration of 0.5 msec given either singly or as tetani controllable in frequency and duration.

In newborn and young kittens the fibres in the gastrocnemius nerve are very thin. As shown by SKOGLUND (1960 b) the conduction velocity of the afferents is only about 10 m/sec in the newborn kitten suggesting (and confirmed) in a publication in course of preparation (SKOGLUND and VALLBO 1960), that the fibres are only around 2—3 μ in diameter. Furthermore it has been found difficult to obtain proper fixation of the limb because the bones are very soft. Difficulties of fixation in combination with failure to denervate all muscles around the hip and spine for fear of disturbing the fibres running to distal muscles sometimes introduced difficulties of identification from which muscle a response was obtained. The procedure adopted was to put aside the filaments recorded from, to retest them later after section of the gastrocnemius nerves, when the response had to be abolished.

By this method very few sense organs could be studied in one and the same animal. The experiments were also restricted to a short periods of observation. This was found to increase validity of the results because muscle receptors and the muscle itself deteriorate during longlasting experiments. Temperature of the muscle and its blood supply are of great importance for the maintenance of good function of muscle spindles (GRANIT and HOMMA 1958). In dissecting, great care was taken not to disturb its blood supply but when freeing the muscle from overlying skin it might have been slightly injured. The temperature of the muscle has been continuously controlled by a small thermocouple put under the skin, and when necessary the muscle was warmed by lamps and the whole limb wrapped in cotton wool.

It might be argued that the weak or absent tonic properties of muscle receptors in the newborn and young kitten, to be described under Results, are due to disturbances such as those mentioned above. However, the tonic discharge from muscle receptors obtained only some days after birth under identical experimental conditions is a fact which contradicts this criticism.

A possible methodological error, to be taken up further under Results, is the relatively small threshold difference between alpha and gamma fibres in the young kitten (SKOGLUND 1960 b). A slightly supramaximal stimulation of the alpha fibres might activate some gamma efferents and so make differentiation of fibre types difficult. In the complete absence of effects on the muscle spindles of efferent nerve stimulation this state of affairs is of course no trouble. When tetanizing the immature nerve fibres they easily become blocked unless very strong stimuli be used, probably owing to summation of positive afterpotentials, which by HURSH (1939) have been shown to be of greater magnitude in immature fibres. Therefore, when stimulating the gamma efferents, shock strengths of 10 times for the alpha threshold were used although a late wave, probably corresponding to the gamma wave in the adult animal in the kitten appears at shock strength 5 times threshold for the alpha fibres (SKOGLUND 1960 b). Rather short tetani have also been used to avoid the cumulative effect of positive afterpotentials when tetanizing. The short duration of the tetanus was compensated for by using high frequencies (cf. KUFFLER, HUNT and QUILLIAM 1951).

Results

Muscle receptor responses to stretch and succinylcholine. When recording from an isolated dorsal root filament or filament of the peripheral nerve belonging to the gastrocnemius muscle of a newborn or young kitten, extension of the muscle never gives rise to the characteristic well sustained spindle discharges, but only to the very weak tonic responses, illustrated in Fig. 1 A from a 3 days old kitten. Yet (Fig. 1 B) repeated stretch always elicits a brief response. Some-

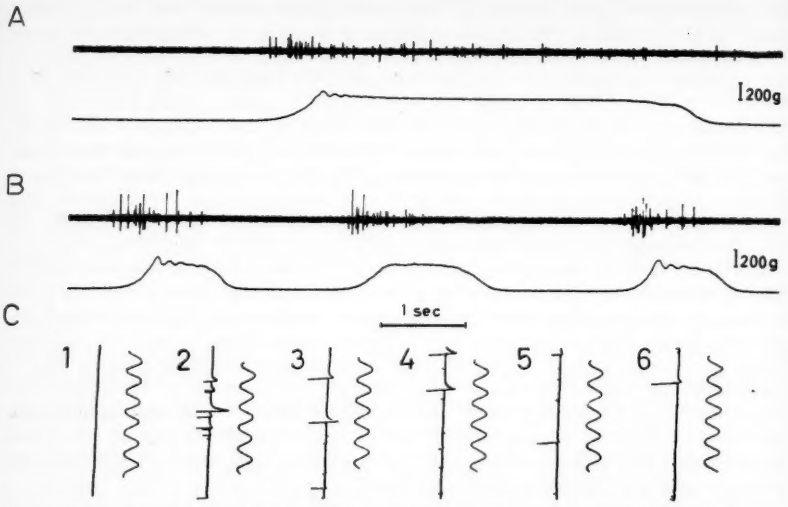

Fig. 1. The response of muscle receptors in the gastrocnemius muscle of a 3 days old kitten recorded in a thin filament of the dorsal root S 1. A response to an 8 mm stretch of the muscle kept at constant length until released. Note, falling tension and faint, irregular discharge. In B, three short pulls of the muscle set up a discharge each time. C, response recorded on fast sweeps at 3 sec interval. In record 1 the receptors are silent during a muscle tension of 60 g. Then 20 μg succinylcholine injected and from record 2 on bursting responses occur. Spikes retouched. For further comments see text.

times a sense organ may fire a few impulses in succession but usually it quickly adapts and is silenced. It has often been noticed that muscle receptors, obviously situated in proximal muscles which are difficult to denervate, respond with well sustained discharges to constant pressure at a time when such responses never can be obtained from the distal muscles of the newborn kitten.

In the beginning of the work sustained discharges set up stretch were often erroneously attributed to the distal muscle under investigation. On account of the difficulties of identification (see Methods), it was decided to attribute to the distal muscle only those particular responses that disappeared after section of the nerves to the gastrocnemius.

The absence of sustained discharges from the gastrocnemius muscle receptors in response to stretch often made differentiation of the sense organs by contraction (MATTHEWS 1933) difficult, the more so as a discharge from proximal muscles might interfere. Furthermore, owing to differences in conduction distance and the long conduction times (SKOGLUND 1960 b) 'false' proximal responses might appear anywhere within the contraction recorded from distal muscles. However, succinylcholine is known to excite spindles exclusively (GRANIT, SKOGLUND and THESLEFF 1953). By using this drug, given

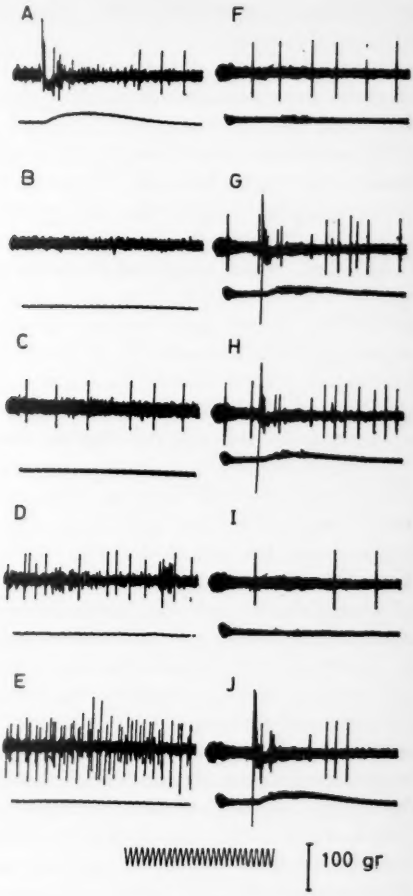

Fig. 2. Records to show the effect of succinylcholine on the muscle spindles in the gastrocnemius muscle in a 4 days old kitten. A, response of the receptors to contraction set up by stimulation of the ventral roots L 7 and S 1. B, the receptors are silent when muscle is under tension of 80 g, which gradually declines during the experiment. Then 50 μg succinylcholine is given in the contralateral femoral artery and the receptors are seen to be activated in C. For further explanation see text. Time 50 c/sec.

intra-arterially in doses of 10—50 μg, differentiation of the responding receptors was possible. In the newborn kitten succinylcholine never set up any longlasting activation of the muscle spindles. As illustrated in Fig. 1 C, only bursts of activity were obtained. However, the sensitization of the spindles by subparalytic doses of succinylcholine sufficed to make them discharge on the falling phase of the contraction in the newborn animal which they later on do without this drug. Thus, by combining succinylcholine sensitizing the spindles to respond on the falling phase and nerve sections causing this response to disappear, it could be proved that muscle spindles were amongst

the phasically responding sense organs which in the newborn kitten were activated by stretch.

In Fig. 2, from a 4 days old kitten, in which no sustained discharges to stretch of the gastrocnemius muscle could be obtained, as seen in B, stimulation of the ventral roots caused firing of some Golgi organs on the rising phase of the contraction and of one muscle spindle on the falling phase as seen in A. These sense organs were all situated in the gastrocnemius as controlled after the experiment. A small dose of succinylcholine excites the spindle to set up a discharge which in the beginning was regular (about 15 imp/sec) as seen in C but then, while increasing in frequency, became irregular, as seen in D and E. When later on in F the effect of succinylcholine faded off the frequency (about 20 imp/sec) again became regular. On account of the blocking effect of succinylcholine, stimulation of the ventral roots in G and H set up a smaller contraction than in A, but the sense organs firing on the falling phase of the contraction responded with more spikes than in A. Then, in I, the frequency fell even more and in J the response was about the same as in A.

Succinylcholine which also acts directly on the muscle spindle (GRANIT *et al.* 1953, HENATSCH and SCHULTE 1958) can thus force the sense organs to fire in the newborn animal with bursts of impulses (see Fig. 1 C) and in older animals with a sustained discharge which, however, becomes irregular at high frequencies (see Fig. 2). Actually GRANIT *et al.* (1953), when using high doses of succinylcholine, saw spindles that stopped firing after a brief high frequency discharge. The present experimental results, however, point to some property of the receptor terminal in the newborn kitten, which prevents high frequencies of firing and this might be one cause of the failure of the spindles to set up sustained discharges.

With increasing age of the kittens better sustained discharges from sense organs in the gastrocnemius muscle are obtained in good preparations though still at low frequencies. As stated in an earlier publication (SKOGLUND 1960 a) neither body weight nor postnatal age are good measure of neural maturity. It was therefore decided to correlate the response of muscle receptors with the conduction velocity of the peripheral nerves. In doing so it has invariably been found that the conduction velocity of the afferents have to reach about 16—18 m/sec before any well-sustained discharges are obtained from the muscle spindles.

It might be asked if the nerve fibres below a conduction velocity of 16—18 m/sec have any peculiar properties. HURSH (1939) determined the absolute refractory period of skin nerves during development and found it to be twice that of the adult nerve at a conduction velocity of 10 m/sec. This has been confirmed and found to hold good for the muscle afferents too. As illustrated in Fig. 3, where the afferents of the gastrocnemius nerve are conditioned with a supramaximal shock and then tested with a shock 3 times that strength, the relative refractory period is more than 10 msec and the absolute

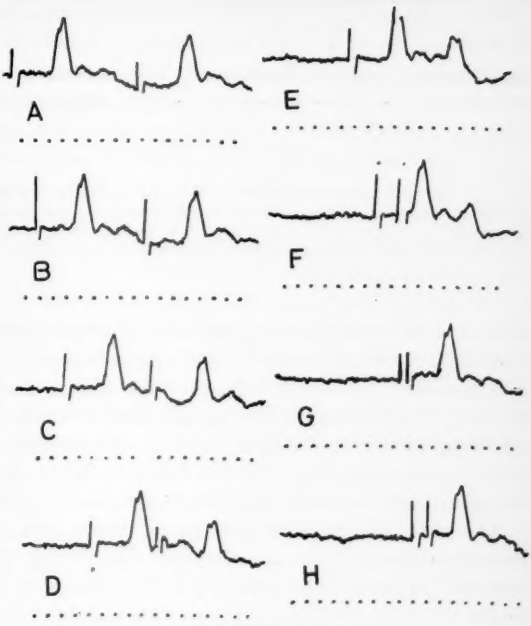

Fig. 3. Records to show the duration of the absolute and relative refractory periods of the afferents in the gastrocnemius nerve of a newborn kitten. Conduction velocity of the fastest fibres measured to 11 m/sec. The conditioning shock was well supramaximal and the test shock 3 times that strength. In A can be seen that the relative refractory period exceeds 10 msec. Then the conditioning shock is shifted towards the test shock in the records from B—G. Note, increase of latency between shock artefact and test response clearly seen in C and D. In F a very small response is obtained with 2 msec interval between conditioning and testing and in G the test response has disappeared completely at 0.8 msec between the shocks. In H the response has not reappeared although conditioning and testing are separated by 1.2 msec. Time in msec indicated by dots. For further comments see text.

refractory period more than 1 msec. HURSH (1939) has clearly shown that the absolute refractory period decreases with increasing conduction velocity to reach adult values at a conduction velocity of 20 m/sec of the fastest fibres in the saphenous nerve. This has been found to be true for the muscle afferents too.

Since the absolute refractory period in the immature nerve is of longer duration than in the adult one the capacity of the nerve fibre and the receptor terminals for conducting at high frequencies will also be restricted (KATZ 1950). HURSH (1939) has further shown that the immature nerve fibres have a positive afterpotential of longer duration and greater magnitude than mature fibres. This is one more restriction on the absolute value of rate of firing; the fibres are blocked owing to hyperpolarisation created by summation of positive afterpotentials.

An inability of the immature nerve fibres to conduct repetitively, at least at high frequencies, might be one explanation of the lacking tonic discharge from muscle receptors in response to stretch and depolarising agents. There is, however, another factor which similarly tends to cut short a tonic discharge in the newborn and young kitten. In Fig. 1 A can be seen that the muscle

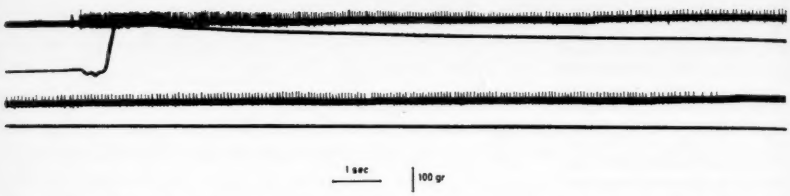

Fig. 4. The response of some muscle receptors in the gastrocnemius muscle of a 10 days old kitten recorded in a filament of the dorsal root L 7. The records are in direct continuation. The muscle is extended 10 mm and kept at constant length. Note fall of tension and adaptation of responding receptors. For further explanation see text.

tension starts to decrease immediately although the muscle is kept at constant length. This viscoid reaction of the muscle leading to compliance in stretch was described by BLIX (1893) and further shown by LANGLEAN (1915) to be due to a lengthening of the muscle under constant load. No systematic investigation has hitherto been made of the compliance of muscle during postnatal development, but it is here found to be of a considerable magnitude in the gastrocnemius of the newborn kitten and decreases with increasing age.

The effect of compliance on the discharge of a muscle spindle is demonstrated in Fig. 4. The gastrocnemius muscle in a 10 days old kitten was extended 10 mm and kept at constant length. A few sense organs start firing in the beginning of the upper record but most of them soon drop out so that in the lower record which is in direct continuation there is only one left. It, too, ceased firing in the end. This is best explained by compliance adding to sensory adaptation and owing to which tension diminishes to less than half the initial value in the course of the record.

In the experiments it was often tried to compensate for this yield of tension by increasing extension. To some extent this could be done but the sense organs, at the new muscle length, usually stopped firing rather soon. In the newborn and very young kitten it was thus not possible to obtain a lasting discharge in that way. In this respect the receptors differed a great deal from those of grown-up animals which fire with but little adaptation as long as stretch is maintained. Whether the adaptation at increased muscle length is entirely due to greater yielding of the muscle fibres cannot be stated. Alternative explanations are that the firing of the spindle is prevented in the receptor terminals or that extensions which must be regarded unphysiological, sometimes amounting to over 15 mm, lead to deterioration of the receptor structure whereby the discharge is abolished. Increasing the length of the muscle in this way often resulted in complete failure of the end organ to respond to stretch. This favours the latter explanation. It has, however, often been found that after release of a large extension during which the sense organs adapted to silence, fresh extension again evoked a discharge adapting

to silence even when loss by compliance continually was compensated for. This evidently points to certain properties of the receptor terminals which, in combination with the increased compliance of the immature muscle, are responsible for the phasic behaviour of its receptors. Separation of these factors certainly deserves further experimentation. Another feature of the immature muscle is its slow contraction described by DENNY-BROWN already 1929. This phenomenon has been studied but will not be taken up until in a later work on the efferent link of the stretch reflex.

The general experience of the Golgi organs in the newborn animal were that they behaved like the muscle spindles with regard to constant stretch of the muscle. On the other hand they seemed rather easily activated by contraction indicating that their mode of action in the early stages is phasic.

The activity of muscle receptors during contraction and gamma fibre activation. Differentiation of muscle spindles and Golgi tendon organs before a sustained discharge was obtained in response to stretch was carried out with the aid of succinylcholine. This drug would sensitize an otherwise completely silent spindle so as to make it fire some spikes on the falling phase of the contraction and thus show up the pause.

The muscle spindles begin to give tonic discharges in response to stretch 6—10 days after birth. However, even these better sustained discharges adapt quickly, probably on account of the great compliance of the immature muscle (see Fig. 4). When, with increasing age of the animal, the response becomes better sustained, the muscle receptors can be investigated in relation to development of tension (MATTHEWS 1933). It was then attempted to fill in the pause in the discharge of a muscle spindle by stimulation of its gamma efferents. (LEKSELL 1945, KUFFLER *et al.* 1951). It has earlier been shown (SKOGLUND 1960 b) that after ten days a late wave is obtained in the motorneurogram of the gastrocnemius nerve on stimulation of the ventral roots at 5 times alpha threshold. The development with age of the conduction velocity of the fastest fibres in this late wave makes it highly probable that it corresponds to the gamma wave in the motorneurogram of the adult animal (SKOGLUND 1960 b).

In Fig. 5 A is seen the response of a muscle spindle in the gastrocnemius muscle under moderate tension in a 12 days old kitten. In B and C the ventral roots L 7 and S 1 are stimulated with a short tetanus using a shock strength that gave a maximal muscle contraction. In D—F the shock strength was increased to ten times threshold for the alpha fibres. As is seen there is no filling in on the pause during contraction. On the falling phase, however, a few spikes are added. If these spikes really come from the same sense organ could not be ascertained with certainty.

In spite of stimulation of the ventral roots at shock strength high enough to set up a late wave in the neurogram, the pause in the discharge of a muscle spindle during contraction has never been filled in before 17—20 days after

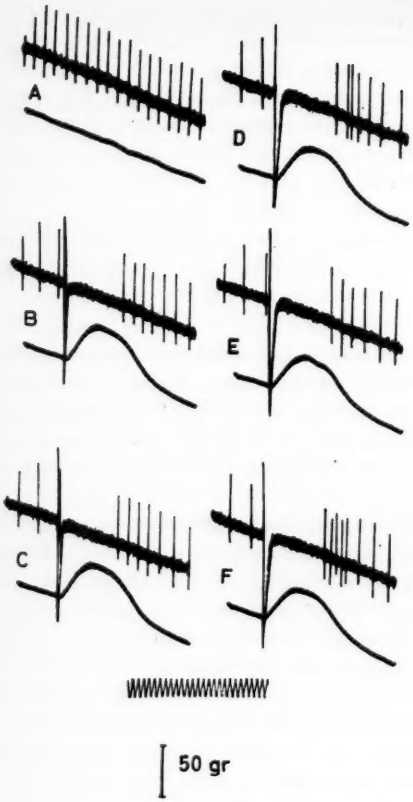

Fig. 5. The response of a gastrocnemius muscle spindle in a 12 days old kitten during muscle contraction and gamma efferent stimulation. A, discharge immediately after onset of an 8 mm stretch; frequency 20 imp/sec. This response gradually adapted to around 12 imp/sec. B and C, ventral roots L 7 and S 1 are stimulated with 4 shocks at a frequency of 180/sec at strength maximal for alpha fibres. In D—F shock strength increased by 10 times, other conditions unchanged. Time 50^{c}/sec. For further comments see text.

birth. This statement is based on experiments in which both the initial tension of the muscle and the duration and frequency of the stimulation have been varied over wide ranges. As shown by KUFFLER *et al.* (1951) these factors are of great importance for the demonstration of gamma control over the muscle spindles.

Initially many mistakes were made in this work, because effects of gamma efferent stimulation on receptors in proximal muscles were often erroneously attributed to the muscle spindles in the distal muscle under observation. However, section of the muscle nerve showed up such errors beyond doubt. Fig. 6 is from a 14 days old kitten and in A is seen the tonic discharge of a muscle spindle in the gastrocnemius muscle under moderate initial tension. In B the sense organ pauses during a contraction of the muscle set up by stimulation of the ventral roots with a tetanus of short duration at shock strength maximal

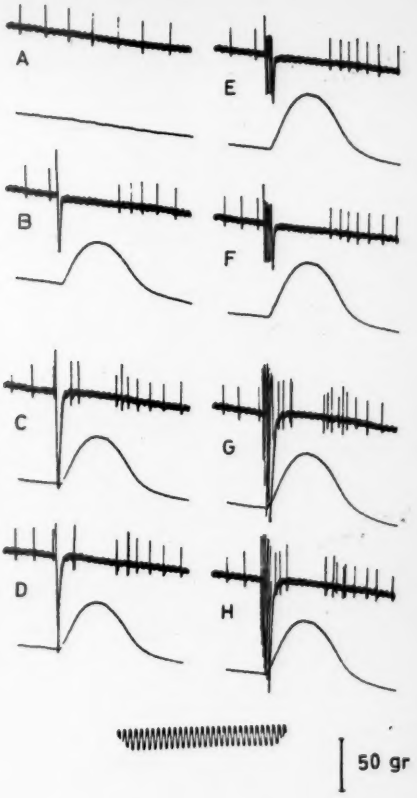

Fig. 6. Records to show gamma activation of muscle spindles in proximal muscles of a 12 days old kitten. In A is seen the adapted response of a gastrocnemius spindle under moderate tension of the muscle. B, ventral roots L 7 and S 1 stimulated with 4 shocks at a frequency of 250/sec at strength maximal for alpha fibres. C and D, shock strength increased to 10 times threshold for the alpha fibres. E and F, shock strength reduced again to maximum for the alpha fibres but duration of tetanus increased (8 shocks at freq. 250/sec) G and H, shock strength again increased to 10 times threshold. Time 50c/sec. For further comments see text.

for alpha fibres. In C and D the short tetanus on the ventral roots is increased in strength to ten times threshold for the alpha fibres and now some spikes appear on the rising phase of the contraction and one is also seen to fire on the falling phase. In G and H the same experiment is repeated with a tetanus of longer duration and the same outcome. E and F being the controls at stimulus strengths maximal for the alpha fibres. Judging from the size of the action potentials, the additional spikes appearing in response to gamma stimulation must belong to some other sense organ than the one firing continuously. The firing on the falling phase of the contraction exhibited by this new sense organ is typical for a spindle. After section of the nerves to the gastrocnemius muscle the continuously firing spike disappeared, but the spike responding to gamma efferent stimulation could be activated by pressure near the spine. Unfortunately it was soon lost and further study of its properties

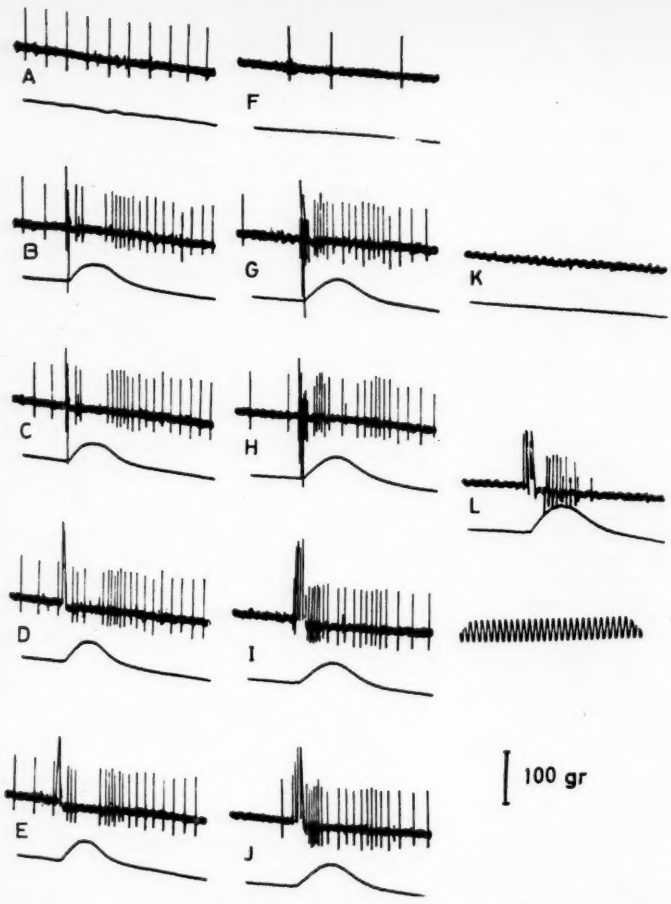

Fig. 7. Records to show alpha control of a gastrocnemius muscle spindle in a 14 days old kitten. A, response immediately after onset of 8 mm stretch. B and C, ventral roots L 7 and S 1 are stimulated with 4 shocks at a frequency of 200/sec at shock strength nearly maximal for alpha fibres. D and E, shock strength increased to 10 times threshold for the alpha fibres. F, the adapted response irregular and slow in contrast to A. G and H, duration of tetanus increased (7 stimuli at freq. 200/sec), shock strength adjusted to maximum for alpha fibres. L, response of some Golgi tendon organs from the same experiment for comparison. K is the silent control. Time 50 c/sec. For further explanation see text.

prevented. Anyhow from this and similar observations it seems that spindles in proximal muscles in addition to setting up sustained discharges earlier than spindles in distal muscles likewise earlier come under gamma control.

Sometimes filling-in of the pause in the discharge of a muscle spindle during contraction was obtained earlier than 17 days after birth, as illustrated in Fig. 7. In A the resting discharge of a spindle in the gastrocnemius muscle under light tension is seen. In B and C the ventral roots are stimulated with a short tetanus at shock strength submaximal for alpha fibres which does not activate gamma fibres and there are seen some spikes on the rising phase of the contraction and an acceleration of the firing on the falling phase. When in D and E the shock strength is increased to ten times threshold for the alpha fibres, very little change in the discharge of the spindle is obtained. In F later on in the experiment the resting discharge has adapted to a lower frequency and it can be seen that the muscle tension has decreased, as indicated by the lower tracing compared with that in A. In G and H the ventral roots are stimulated with a tetanus of longer duration than before but at shock strength maximal for the alpha fibres only. In spite of this the pause is filled in and not much altered when the stimulus strength in L and J is increased to ten times the alpha threshold. That the filling-in is not caused by a Golgi tendon organ is clearly demonstrated by the discharge in L from the same experiment which for comparison shows the typical behaviour of some such organs.

The experimental results shown in Fig. 7 were not at all understood until the publications of GRANIT, POMPEIANO and WALTMAN (1959 a and b) appeared in which they demonstrated alpha activation of the muscle spindles in correspondence with earlier findings of alpha innervation by histologists (CILIMBARIS 1910, GARVEN 1925, BARKER 1948, COOPER and DANIEL 1956). The wellknown demonstration of MATTHEWS (1933) in the adult cat that the pause in the firing of the nuclear bag endings (Matthews type A_2) could be filled in when increasing shock strength by only 10—20 per cent of the alpha maximum may be similarly explained. In the experiment shown in Fig. 7, shock strength could be kept at alpha level to obtain an increased spindle response. We recall that fibre calibres are less differentiated in immature nerves (SKOGLUND 1960 b). In fully grown-up animals contraction velocities of the muscles are also better differentiated but we do not know how intra- and extrafusal velocities are related in kittens. At any rate quite generally the earliest evidence for spindle control in kittens places the mechanism in the alpha range, the more so as it sometimes has been obtained at stimulus strengths slightly below maximum for the alpha fibres (see Fig. 7 B and C). Increase of stimulus strength at this stage of development to include gamma range has failed to influence spindle firing.

After 20 days postnatally differentiation of alpha from gamma efferents can be obtained (SKOGLUND 1960 b). In Fig. 8 is shown an experiment from a 23 days old kitten. In A the gastrocnemius muscle is extended 8 mm and two sense organs start firing, one small and one large. We are only concerned with the large spike. In the beginning of record A the dynamic effect of stretch is clearly seen in the phasic response of the large spike (KATZ 1950), which

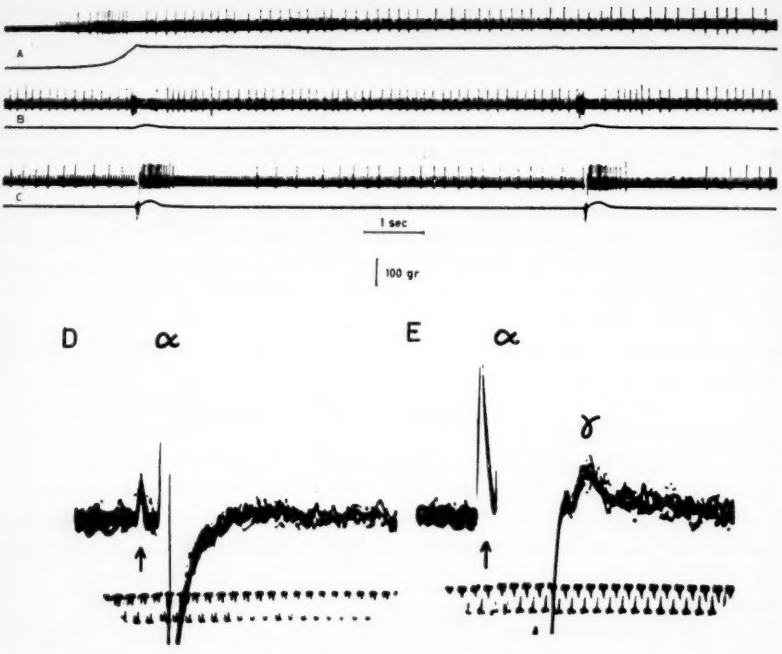

Fig. 8. Records to show gamma activation of gastrocnemius muscle spindle in a 23 days old kitten recorded in a thin filament of the dorsal root S 1. A, muscle stretched 8 mm and kept at constant length. B, muscle tension somewhat increased and ventral roots L 7 and S 1 stimulated twice at shock strengths maximal for alpha fibres (6 stimuli at freq. 150/sec). C, shock strength increased to ten times threshold for the alpha fibres. Myograph sensitivity given in the figure refers to C where it is twice that in A and B. D, alpha wave recorded on the muscle nerve when stimulating the ventral roots at shock strength maximal for the alpha fibres. E, same response on increasing shock strength to the one used in C. Now a late (gamma) wave appears. Arrows indicate shock artefacts. Superposition of 40 faint traces. For further comments see text.

stops firing even before the muscle is fully extended and then starts firing steadily (the static response). This is but an exaggeration of what can be seen in the adult cat (cf. MATTHEWS, 1933). The low frequency of the static response, less than 10 imp/sec, should also be noted. In B the muscle is caused to contract by stimulation of the ventral roots. It is seen that the large spike pauses during contraction. A third even larger spike can now be seen firing occasionally.

Thereafter, in C, the shock strength was increased to ten times threshold for the alpha fibres (giving now the late wave seen in E) and the pause during the contraction was filled in. This shows beyond doubt that the late wave in the motorneurogram corresponds to the gamma wave of the adult stage.

After the falling phase of the contraction the sense organ stopped firing for more than a second. Such postexcitatory depressions which were often seen (or heard in the loudspeaker) after gamma efferent stimulation were described by KUFFLER *et al.* (1951). They found them to last around 200 msec. The duration of the depression could, according to these authors, be decreased by increasing the muscle tension and decreasing frequency and duration of stimulation. No systematic observations have here been made on the effect of varying tension and stimulation but it can be stated that the postexcitatory depressions obtained were of very much longer duration than in the adult animal. KUFFLER *et al.* were inclined to explain this depression by some property of the receptor terminals and according to KATZ (1950) there is a positive potential swing in the afferent terminals on release from stretch while this may be part of the explanation for the phenomenon seen here. Compliance must also play a great role, as suggested by MATTHEWS (1933) (his visco-elastic forces).

Discussion

It has been found above that the muscle receptors in the newborn and young kitten respond more or less phasically to stretch. It might be argued that such responses are due to disturbances in the blood supply (GRANIT and HOMMA 1958) or other abnormalities introduced by the experimental procedure. The objections to such arguments were considered already under Methods. Be this as it may, all experimental observations go to show that the tonic spindle discharge, when present at all in a young kitten is very feebly developed. One feature of it is the low frequency, another that it adapts rapidly, partly at least on account of extensive compliance in the immature muscle. While in the adult stage intense firing follows upon the injection of succinylcholine (GRANIT *et al.* 1953), the discharge in the young kitten was by comparison feebly facilitated. Since succinylcholine effects the sensory terminals, some developmental deficiency in them must be assumed. Lack of maturity involves several properties of the nerve fibre. Thus there is a longer duration of the absolute refractory period in the immature nerve fibre and more hyperpolarisation by summation of positive afterpotentials which are of greater magnitude in the immature fibres (HURSH 1939).

That the electrical properties of the nerve fibre in addition to the mechanical properties of the muscle have something to do with the appearance of a tonic discharge is suggested by the finding that better sustained discharges of the muscle receptors appear about the time when the absolute refractory period of the nerve attains adult values. Such maturation of the nerve fibre need not necessarily be in any way related to the fibre diameter merely because it appears at a certain conduction velocity. The latter value, however, is a convenient reference point.

The importance of the mechanical properties of the muscle for the main-

tenance of a sustained discharge from the receptors has been pointed out. The high compliance of the immature muscle leads to decrease of pull on the nuclear bag and thus corresponds to extrafusal shortening. Further work is, however, needed to ascertain the full significance of the mechanical properties of the muscle for the initiation and maintenance of muscle receptor responses during development. The importance of the mechanical properties of the muscle fibres for the response patterns of muscle spindles has recently been emphasized by GRANIT and HOMMA (1958) and GRANIT, HOMMA and MATTHEWS (1958).

As shown in many papers (for references see BARRON 1941) there is a cranio-caudal and a proximo-distal development of the muscles on the trunk to judge from their reaction to direct electrical stimulation. Probably such a development of the mechanical properties of the muscles also takes place. In a publication under preparation (SKOGLUND and VALLBO 1960) evidence will be presented that there is a cranio-caudal and a proximo-distal development of the nerve fibres to judge from their diameter. It has here been found that sustained discharges seem to appear earlier in the proximal muscles around the hip and spine than in distal muscles. It will remain for further studies to settle these questions definitely but such a scheme of the development would fit well into the demonstrations of SKOGLUND (1960 a) that there is a proximo-distal development of the stretch reflex in the limbs and that it develops earlier in the forelimbs than in the hindlimbs.

The postnatal development of a tonic discharge from the muscle spindles in the gastrocnemius muscle some time after birth (6—10 days postnatally), as found here, is quite in accordance with the evidence presented by SKOGLUND (1960 a) that the proximo-distal development of the stretch reflex is confined to its afferent link. The weak tonic discharge from the muscle spindles suffices to elicit a tonic stretch reflex when the tonic ventral horn cells (GRANIT *et al.* 1956) are facilitated in the decerebrate kitten with additional ablation of the anterior lobe of the cerebellum. In this preparation the tonic stretch reflexes come off even in the absence of gamma support of the spindles as shown by MATTHEWS (1958) and POMPEIANO (1959).

The appearance of gamma control of the muscle spindles 17—20 days postnatally is also well in accordance with the demonstration of the first signs of a tonic stretch reflex in the intercollicularly decerebrated kitten at that time and the later appearance of a decerebrate rigidity depending upon stretch reflexes (SKOGLUND 1960 a). According to ELDRED *et al.* (1953) and MATTHEWS and RUSHWORTH (1957) gamma support of the spindles is necessary for the stretch reflexes in such a preparation. It is interesting to note that the gamma efferents, at the time when they now have been found to control the muscle spindles, have reached a conduction velocity around 15—20 m/sec, just as have the afferents when the tonic discharges appear (cf. SKOGLUND 1960 b). The earlier appearance of gamma support of muscle spindles in proximal

muscles suggests that these muscles probably have more developed nerve fibres.

There might be alpha innervation of the spindle preceding gamma control during development. The latter ought, however, to be weak in the beginning. The failure to demonstrate gamma support of the spindles in the early stages although a gamma wave is obtained in the motorneurogram (SKOGLUND 1960 b) might be due to inability of this slow mechanism of control to facilitate the response by increasing the muscle tension in the face of compliance of the muscle. As mentioned earlier, the muscle tension has by KUFFLER *et al.* (1951) been shown to be of great importance for the demonstration of the gamma innervation of the spindles. Thus gamma activity may in reality exist somewhat earlier than found here.

A weak tonic discharge from the muscle spindles themselves in combination with a less efficient gamma drive offers a good explanation for some phenomena observed in the intact animal when it starts walking on its hindlimbs around a fortnight after birth. As described by SKOGLUND (1960 a) the first walking trials are accompanied by an intentional tremor of slow frequency. This could mean that when the muscle contracts and the spindles are unloaded in the presence of weak or lacking gamma support they would soon be silenced. As a consequence the stretch reflex diminishes and the spindles firing anew when pulled upon as the contraction weakens. In this way the observed tremor would be selfgenerating and be nothing else than the clonus described by GRANIT (1959). This mechanism might of course have central counterparts like recurrent inhibition and rebound as suggested by GRANIT (1959).

A sudden loss of tone which makes the gait uncertain and atactic is a conspicuous feature when the kitten walks around three weeks postnatally (SKOGLUND 1960 a). This phenomenon can of course be explained by weak gamma discharge but the frequently seen postexcitatory depression (illustrated in Fig. 7) for more than a second after gamma activation would also account for loss of tone. It might be argued that the compliance of the muscle could be the direct cause of such losses of tone. This is very unlikely because the loss of tone seems to be a fast affair which compliance is not. Furthermore it must be remembered that already in the newborn kitten pinching of the tail gives an escape reaction when the kitten rises on its feet and is then well able to support its body weight (WEED 1917, SKOGLUND 1960 a). On the other hand compliance might play a role indirectly by causing a diminution of the spindle discharge. This diminution, however, must also be slow.

The gradual appearance of a tonic discharge from the muscle spindles and the late development of their gamma support is in itself a good explanation for the gradual appearance of tonic stretch reflexes in alpha rigidities and the later appearance of tonic stretch reflexes in the intercollicularly decerebrated animal respectively. In publications to follow (SKOGLUND 1960 c and d) it will, however, be shown that this development is not without central counterparts necessary for tone.

Summary

The postnatal development of the discharge from muscle receptors in the gastrocnemius muscle has been studied in kittens from birth to 45 days of age. The postnatal appearance of efferent control over the muscle spindles has at the same time been investigated. Occasional recordings from muscle receptors in proximal muscles around the hip and spine have also been studied.

1) It is found that the response from the stretch receptors in the gastrocnemius muscle tends to be phasic in the newborn and young kitten. Tonic discharges have occasionally been obtained from proximal muscles.

2) By using succinylcholine iodide which activates muscle spindles selectively it has been possible to prove that both these end organs as well as Golgi tendon organs are represented among the receptors that respond phasically to stretch.

3) With increasing age of the animals better sustained discharges are obtained from the receptors in the gastrocnemius muscle (6—10 days postnatally). Evidence is presented that both the maturity of the nerve fibres, as shown by their electrical properties and the mechanical properties of the muscle are of importance for the appearance of tonic discharges from the muscle receptors.

4) After the appearance of better sustained discharges from the muscle receptors their reaction during muscle contraction and gamma efferent activation has been investigated. From occasional recordings of activity in proximal muscles evidence has been found that the spindles in these muscles can be influenced by gamma activation earlier than those in the gastrocnemius muscle.

5) From 17 days on postnatally the gamma control of muscle spindles in the gastrocnemius muscle could be demonstrated. Evidence is presented to the effect that alpha innervation of the spindles becomes active earlier than the gamma support.

6) The significance of the experimental findings presented here is discussed in the light of earlier results on the postnatal appearance of tonic stretch reflexes, decerebrate rigidity and locomotion.

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Central Connections and Functions of Muscle Nerves in the Kitten

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Abstract

SKOGLUND, S. *Central connections and functions of muscle nerves in the kitten.* Acta physiol. scand. 1960. *50.* 222—237. — In this paper the postnatal development of the afferent terminals of muscle nerves is studied by means of the two-neuron arc, in kittens from birth to 45 days. The experimental findings support earlier histological evidence for a postnatal development of the dorsal root collaterals of muscle nerves. Evidence is also presented that the afferent terminals in the newborn and young animals have properties different from those in the adult animal. Facilitation is found to be lacking in the newborn animal while inhibition of extensors from flexors has been found extremely potent and longlasting. The results presented throw new light on earlier findings regarding the postnatal development of postural reflexes.

In a preceding paper (SKOGLUND 1960 a) the postnatal development of the activity of the muscle receptors and of the gamma support of the muscle spindles in the gastrocnemius muscle were found to provide an explanation for the slow postnatal development of the tonic stretch reflexes, as described by SKOGLUND (1960 b). The tonic stretch reflexes in the extensors of the hindlimb do not appear until 10—12 days after birth, when studied under the most unfavourable conditions of gamma activation of the muscle spindles, namely in the so-called alpha animal. This suggested a postnatal development of the afferent link of the stretch reflex from muscle spindles to ventral horn cells because alpha rigidity as well as crossed extensor reflexes (which do not depend on spindles) could be demonstrated already in the newborn kitten (SKOGLUND 1960 b). Fundamentally, therefore, the reflex effector side was functioning.

A delayed development of the discharge from the muscle spindles has actually been found (SKOGLUND 1960 a). In addition there might be a delayed development of the afferent terminals in the spinal cord, for which the results to be described below offer some evidence.

TIEGS (1927) in an investigation of the boutons on the ventral horn cells, found very few in the spinal cord of the newborn and young kitten. WINDLE (1930) described the development of nerve fibre density in the spinal grey matter and correlated it to the reactions of the kitten and stated that nerve fibre density is very much greater in the cervical than in the lumbar region of the cord in a newborn kitten and that it reaches about the same level in both regions 2 months after birth. He also reported that there are very few fibres in the ventral horn in the newborn animal and, furthermore, that the longitudinal fibres in the grey matter likewise are few. REXED and SOURANDER (1960), studying by the Nauta technique the degenerating fibres after dorsal root section in young animals, observed that the number of degenerating dorsal root collaterals directed to the ventral horn seem to increase with increasing age of the animal.

Thus there is morphological evidence for postnatal development of the afferent terminals to the ventral horn. The histological technique cannot, however, tell whether or not the existing fibres also function. The conclusion that there is an actual increase of the nerve fibres presupposes that the immature and therefore thin dorsal root collaterals have the same stainability as the adult fibres and can only refer to fibres seen in light microscopy. The morphological approach is of course the only way whereby an actual increase in the number of nerve fibres can be demonstrated. By the electrophysiological method, however, some information can be obtained about functional connections of dorsal root collaterals during development. Also their properties can to some extent be studied.

In an earlier publication (SKOGLUND 1960 c) it was shown that the afferents from the muscle spindles in the hindlimb have established monosynaptic connections with the ventral horn cells already at birth, as suggested by MALCOLM (1955). In the present paper the monosynaptic potentials elicited from various muscle nerves in the hindlimb of the kitten have been used for studying the central connections and functions of the dorsal root collaterals. Furthermore the size and recovery curve of the monosynaptic potential has been used for studying the effect of antidromic activation. The postnatal development of facilitation and inhibition has earlier been studied by MALCOLM (1955) and his principal results have been confirmed.

Material and Methods

The experiments to be reported have been performed on kittens from 70 different litters ranging in age from birth to 45 days. In all, some 80 animals have been used. Most of the animals were anesthetized with Nembutal (Abbot) 20—30 mg/kg body

weight given i.p. All experimental results have been confirmed in decerebrated and spinalized decerebrated animals. With the light Nembutal anesthesia used no differences could be observed between the anaesthetized and decerebrated animals with regard to the two neuron arc studied. Well maintained respiration was essential for activity, and any failure in this regard led to diminution of the reflexes recorded. Very often restoration of the synaptic activity was easily obtained with artificial respiration. The temperature of the animals, continuously controlled by thermocouples, was kept at 37—38° C by placing them on a heated table and warming them by lamps. All dissected nerves were kept in paraffin pools at 37° C. It has been found that breathing and body temperature are difficult to preserve in the newborn and small kittens while on the other hand the heart very seldom fails and often far outlasts respiratory arrest.

Lumbar laminectomy was usually performed and either all ventral or all dorsal roots from L 2 and downwards were severed. Monosynaptic potentials were set up by electrical stimulation of either dorsal roots or muscle nerves and recorded either on muscle nerves or ventral roots. The nerves used were those to gastrocnemius, tibialis anticus, biceps-semitendinosus and quadriceps, except the branch to its rectus portion. All muscles in the leg were denervated and the ilio-psoas muscle cut.

Antidromic conditioning shocks were applied to peripheral nerves or to ventral roots. The stimuli were square wave shocks of 0.3—0.5 msec duration delivered through shielded transformers. The electrodes were Ag—AgCl hooks. Monosynaptic potentials were recorded in the conventional way and displayed on one beam of a double beam cathode ray oscilloscope.

A methodological limitation in studying the reflex effects of muscle nerves in the kitten is that no differentiation between fibres with different reflex effects can be achieved by electrical stimulation. The reason for this is that all fibres seem to have about the same conduction velocity at birth and develop at different rates postnatally (Skoglund 1960 c). Thus only with increasing age of the animals the fibres spread out in a calibre spectrum with consequent differentiation of electrical thresholds. In this paper, in stimulating muscle nerves and roots, it has only been possible to use stimuli 1) too weak to elicit a monosynaptic reflex, 2) slightly suprathreshold for the monosynaptic reflex, 3) supramaximal for the monosynaptic reflex and 4) supramaximal for the stimulated nerves or roots.

Results

Analysis of the effects set up in a monosynaptic pathway by its activation.

In the newborn kitten, following a monosynaptic reflex from a dorsal root or a muscle nerve, there ensues a long period of hypoexcitability to subsequent stimulation, confirming Malcolm (1955). Fig. 1 illustrates the effect on the monosynaptic potential recorded from the cut ventral root S 1 after homosynaptic conditioning of the nerves to gastrocnemius (Brock, Eccles and Rall 1951) with a shock maximum for the monosynaptic response. All ventral and all dorsal roots from L 2 downwards were severed except the dorsal roots L 7 and S 1. Testing was carried out with a shock supramaximal for the nerve and the result is plotted in per cent of the control value (100 %) on the ordinate against two different time scales in A and B on the abscissa. As seen in A, a small initial response is obtained 2—10 msec after conditioning, then follows complete unresponsiveness for a hundred msec. The reflex recovers to

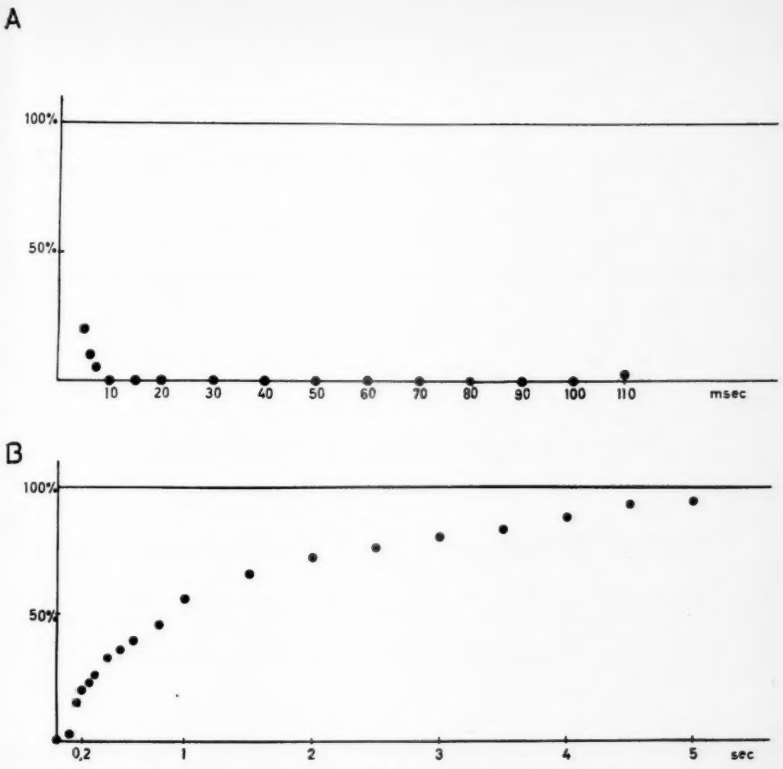

Fig. 1. 1 day old kitten anesthetized with Nembutal. The recovery of the monosynaptic test response set up from the gastrocnemius nerve in a newborn kitten after homosynaptic conditioning at shock strength maximal for the reflex. All ventral and all dorsal roots from L 2 and downwards severed except the dorsal roots L 7 and S 1. The size of the test response is plotted in per cent of the control value (100 %) on the ordinate and interval between conditioning and test shock on the abscissa. The dots are averages of ten values. A, early recovery with a small response 2—10 msec after conditioning followed by complete unresponsiveness up to 100 msec. B, late phase of recovery. Note different time scale in A and B and that recovery is not completed at 5 sec.

about 50 % of its control value in 1 second. The normal state of affairs (in B) is not completed until after 7 seconds or more. By comparison with similar measurements in the adult animal by BROOKS, DOWNMAN and ECCLES (1950 a) the time course of recovery is found to differ in many ways. The early phase, during which there is a very small response in the newborn kitten, differs in two respects from that in the adult animal. In the first place there is not the early facilitation of BROOKS *et al.* (1950 a). Sometimes a small response is obtained, as in Fig. 1, 2—10 msec after the conditioning volley but often there

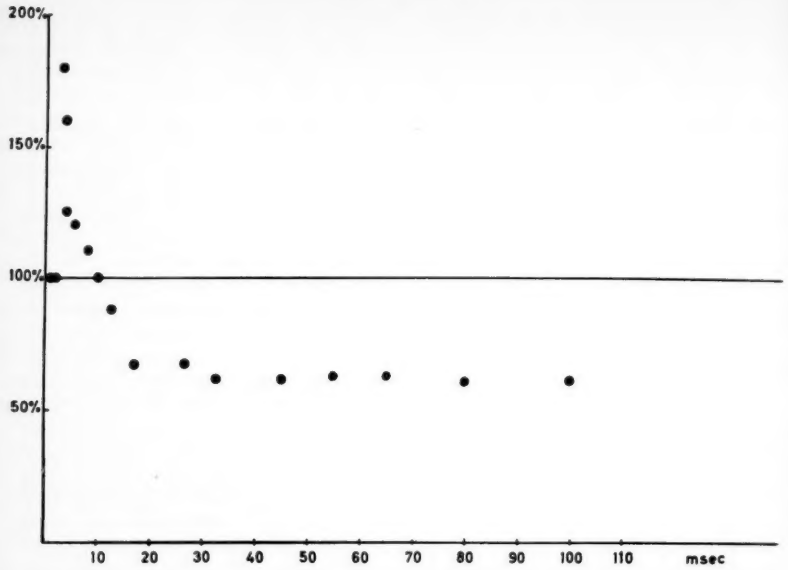

Fig. 2. Same animal as in Fig. 1. The monosynaptic test response from gastrocnemius after homosynaptic conditioning at shock strength slightly subthreshold for the reflex. All ventral and all dorsal roots from L 2 and downwards are cut except the dorsal roots L 7 and S 1. The size of the reflex plotted as in Fig. 1. The dots are averages of ten values. For further comments see text.

is no response whatever. Secondly, this period of recovery is very much longer than in the adult animal where it amounts to only 40—60 msec according to BROOKS *et al.* (1950 a).

When the monosynaptic reflex is conditioned by a very weak shock which does not evoke any recordable reflex response a brief early facilitation and a later depression are obtained (Fig. 2) just as in the adult animal (cf. BROOKS *et al.* 1950 a).

In Fig. 3 A the monosynaptic response has been tested after supramaximal antidromic conditioning of the motoneurones. The antidromic effect is seen not to be as strongly depressant as in the adult animal (cf. BROOKS *et al.* 1950 b, LLOYD 1951). However, the ventral horn cells which actually have been invaded by the antidromic activation are not blocked for durations any longer than in the adult animal. A shock applied to the root used for testing sets up afterhyperpolarisation (BROCK, COOMBS and ECCLES 1953) as well as recurrent inhibition. The latter factor may be separated by conditioning through one and testing through the other gastrocnemius nerve, provided that the dorsal roots are cut. By this method recurrent inhibition of the motoneurones (REN-

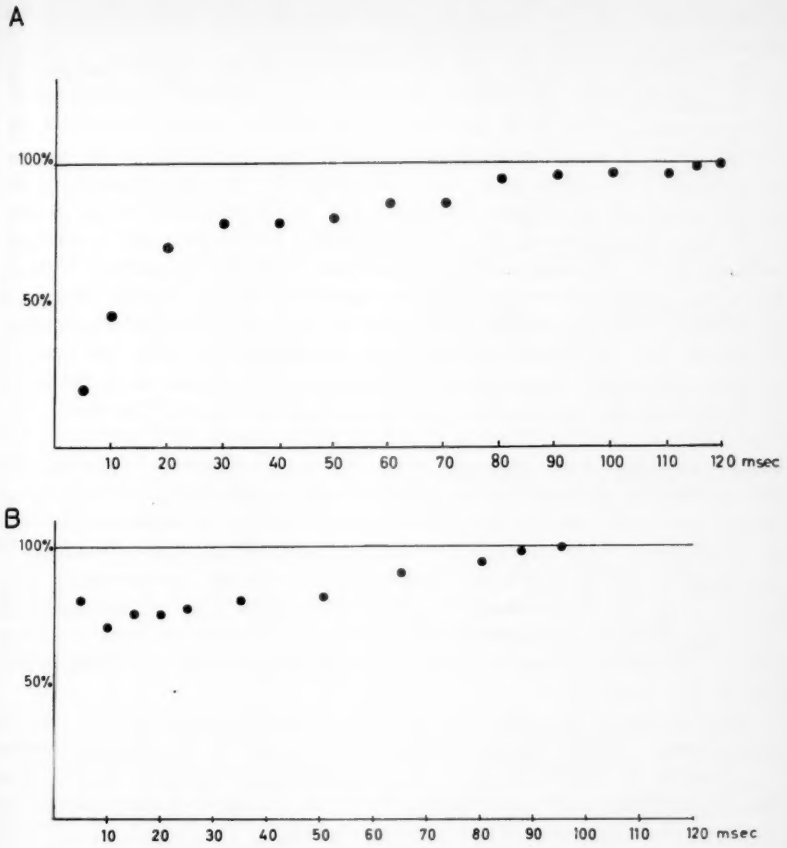

Fig. 3. The effect of antidromic and recurrent conditioning on the monosynaptic potential elicited from the dorsal root S 1 and recorded on the gastrocnemius nerves in a 1 day old kitten. All dorsal and all ventral roots from L 2 and downwards severed except the ventral roots L 7 and S 1. The size of the test response plotted as in the previous figures. A, recovery curve after antidromic conditioning on both nerves to gastrocnemius. B, the effect of recurrent inhibition set up from one of the nerves to gastrocnemius. No significant difference was obtained when the monosynaptic reflex was elicited from both L 7 and S 1 dorsal roots and the conditioning shifted to the other gastrocnemius nerve.

SHAW 1941, ECCLES, FATT and KOKETSU 1954) in the newborn animal was here (see Fig. 3 B) found to be less powerful than in an adult animal under similar experimental conditions (cf. RENSHAW 1941). On the other hand the duration of recurrent inhibition was twice that in the adult animal. This long duration will be discussed later on. After 30 days postnatally both antidromic+

recurrent and recurrent stimulation alone have about the same effects on the monosynaptic potential as in the adult animal.

The experimental results with antidromic stimulation suggest that part of the first phase of recovery of the monosynaptic potential after maximal homosynaptic conditioning in the newborn kitten, like the second slow phase (cf. ECCLES 1953) is attributable to a diminished presynaptic excitatory action. If afterhyperpolarisation alone was responsible for the long depression one would except the afterhyperpolarisation after antidromic + recurrent activation to be more powerful. The lack of an early facilitation after a maximal orthodromic conditioning volley also points to such an explanation. That is, few if any of the afferent terminals are capable of firing a second time in a short interval as they would have to do for early homosynaptic facilitation. The brief early facilitation obtained with a conditioning volley too weak to set up a recordable monosynaptic potential shows that just as in the adult animal, there is a considerable subliminal fringe in the newborn kitten too. The difference in effect obtained with a maximal conditioning volley and one subthreshold for the monosynaptic response seems to allow the conclusion that the terminals of the largest afferents are capable of responding twice in quick succession thereby giving a temporal summation which is hidden in the sum total of events with maximal conditioning. Actually in the experiments of BROOKS *et al.* (1950 a) one can find traces of a similar mechanism, in that conditioning with subthreshold stimulus gives more early facilitation than with maximal conditioning. Anyhow the experiments indicate that the immature dorsal root collaterals have properties different from those in the adult animal (cf. WALL 1958).

With increasing age of the animal the recovery curve after maximal homosynaptic conditioning attains adult features. Around 30 days postnatally the complete recovery is finished in 3—4 sec and there is a clearcut early facilitation at any strength of conditioning. These observations indicate that there is a postnatal development of the afferent terminals.

The postnatal development of facilitation and inhibition of the two-neuron arc in the kitten.

When the monosynaptic reflex from one gastrocnemius nerve is conditioned from the other, no facilitation is obtained between the synergists as in the adult animal (LLOYD 1946 a) until the kitten has reached an age of 10—12 days (cf. MALCOLM 1955). Between synergists working at different joints no facilitation has been obtained either, until that time, and usually even later. These results are quite in accordance with those of MALCOLM (1955) and immedietaly lead to the conclusion that there must be delayed functional development of the dorsal root collaterals postnatally. The lack of facilitatory influences from the muscle nerves seems also to hold true for the

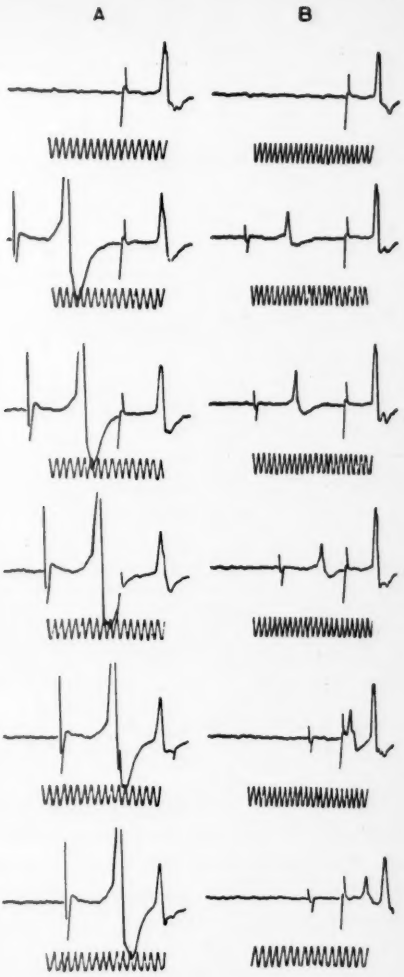

Fig. 4. 1 day old kitten. Records to show lack of late facilitation but presence of early inhibition of tibialis anticus by gastrocnemius conditioning. All ventral roots cut and all dorsal roots from L 2 and downwards severed except L 7 and S 1. *A*, uppermost unconditioned monosynaptic reflex from tib. ant., recorded in ventral L 7. In the following records this reflex is conditioned by a supramaximal shock to the gastrocnemius nerve setting up a reflex response whose monosynaptic component dominates records. No facilitation of the monosynaptic reflex from tib. ant. *B*, uppermost in unconditioned reflex from tib. ant. In the following records this reflex is conditioned by shock to the gastrocnemius nerves slightly above threshold for a monosynaptic reflex. As seen in the last record which is on a faster sweep some inhibition is obtained when conditioning precedes testing by 4 msec. Owing to differences in conduction distances this means an actual difference of 2 msec for the arrival of the afferent volleys to the cord. Time 1,000 c/sec.

Group II facilitation of flexors from extensors (see LLOYD 1946 b), as illustrated in Fig. 4, where in A the monosynaptic potential from tibialis anticus is conditioned by a supramaximal stimulation of the gastrocnemius nerve and no facilitation is obtained. In B the monosynaptic potential of tibialis anticus is conditioned by a stimulus slightly suprathreshold for a monosynaptic reflex from the gastrocnemius nerve. As can be seen in the last record a small early inhibition is obtained.

In contrast to MALCOLM's results (1955) that inhibition does not appear

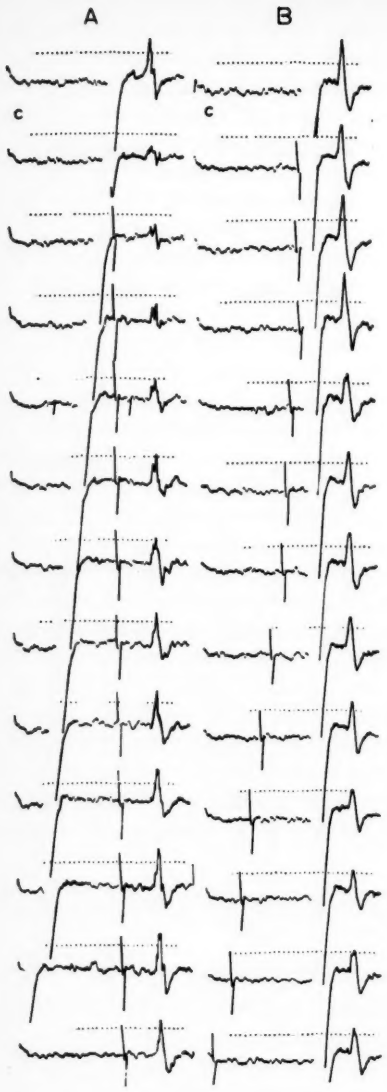

Fig. 5. Records to show early and late inhibition between flexors and extensors in a 4 days old kitten. All dorsal and all ventral roots from L 2 and downwards severed except the dorsal roots L 5, 6 and 7. *A*, c is unconditioned monosynaptis reflex from the combined biceps-semitendinosus recorded in L 7. In the following records this reflex is conditioned by a supramaximal shock to the nerve of quadriceps (except its branch to the rectus portion). Strong early inhibition is seen which diminishes with increasing interval between test and conditioning shock (see artefacts). No late facilitation. *B*, c is the unconditioned monosynaptic reflex from quadriceps (except the rectus portion) recorded in L 6. In the following records this reflex is conditioned by a supramaximal stimulation of the nerves to biceps-semitendinosus. Little if any early inhibition, but with increasing time between the shocks definite inhibition becomes apparent. In both A and B the stimulating electrodes were arranged so that the afferent volleys reached the cord simultaneously. Time 1,000 c/sec. For further comments see text.

until after a few days postnatally it has never been found difficult to produce inhibition of the monosynaptic potential elicited from the gastrocnemius nerves by conditioning from the nerve to the tibialis anticus in a newborn kitten. On the other hand, the amount of early inhibition that can be produced from

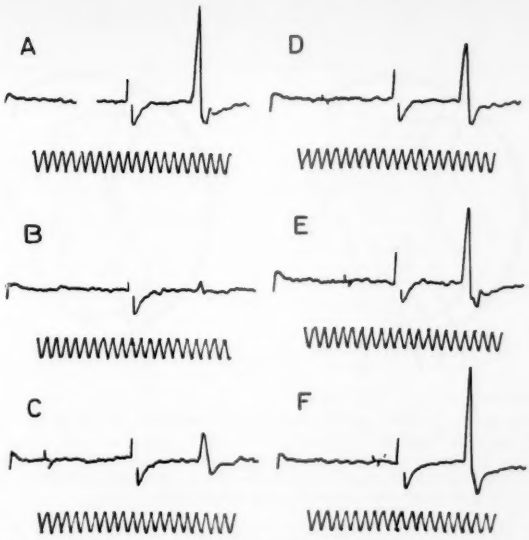

Fig. 6. 3 days old kitten. Records to show late strong inhibition of the gastrocnemius monosynaptic reflex from tibialis anticus. All ventral and all dorsal roots from L 2 and downwards severed except the dorsal roots L 7 and S 1. *A*, unconditioned monosynaptic reflex from gastrocnemius recorded in S 1. *B*, this reflex inhibited by supramaximal conditioning of the nerve to tib. ant. (see shock artefacts in the beginning of sweep). Inhibition diminishes when the time between the shocks is decreased in the following records from C to E and is gone at shock interval in F for which shocks are 2 msec apart. The afferent volleys reached the cord simultaneously. The reflex from tib. ant. could only be obtained in L 7. Time 1,000 c/sec.

one antagonist upon another in the newborn and young kitten is exceedingly variable.

In Fig. 5 A, the monosynaptic reflex from the combined biceps-semitendinosus nerve is conditioned from quadriceps with a stimulus just maximal for its monosynaptic reflex. As can be seen, there is a considerable early inhibition which diminishes with increasing time between conditioning and testing shocks and has disappeared altogether when the conditioning precedes testing by 10—12 msec. No late facilitation is seen. In the series of records shown in Fig. 5 B the monosynaptic potential from quadriceps is conditioned from biceps-semitendinosus with a stimulus just maximal for its monosynaptic reflex. As can be seen the early inhibition is very small if at all present but with increasing time between conditioning and testing shocks a late inhibition appears.

In the experiments illustrated in Fig. 5 the afferent volleys reached the spinal cord simultaneously, when stimulating both nerves simultaneously, so the difference can not be due to any difference in time in the afferent path outside the cord. It has been a constant finding that early inhibition from

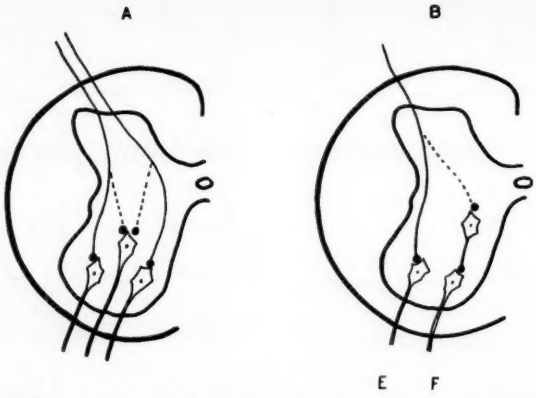

Fig. 7. Schematic drawings of the developing dorsal root collaterals indicated by broken lines. In A the developing collaterals converging on one and the same ventral horn cell which would be the anatomical substratum for the developing facilitation. In B a developing dorsal root collateral is indicated which mediates inhibition via an interneuron (cf. ECCLES, FATT and LANDGREN 1956). E and F denote extensor and flexor respectively.

the extensors on the flexors, as shown in both Fig. 4 B and Fig. 5 A, is stronger in the newborn and young kittens than the reverse inhibition from flexors on extensors seen in Fig. 4 A and 5 B. The Group II facilitation of flexors from extensors is however not obtained until some time after birth. The late inhibition from flexors on extensors, on the other hand, is very strong as shown in Fig. 5 B and further illustrated in Fig. 6 where the monosynaptic potential from gastrocnemius is conditioned with a supramaximal stimulation of the nerve to tibialis anticus. The electrodes were arranged so that the afferent volleys reached the cord simultaneously, when the shock artefacts coincided. No inhibition was obtained until the conditioning preceded testing by 5—6 msec as illustrated in Fig. 6. This is in conformity with the finding in Fig. 5 B.

The small and sometimes lacking early inhibition of flexors from extensors in the newborn and young kittens may be due to slow building-up of inhibition at the membrane. However, the constant finding of a small early inhibition the other way round, was found in Fig. 5 A to be over after 10 msec. Therefore the membrane response can be quite fast and so some immaturity in the corresponding dorsal root collaterals or interneurones must be present. This is diagrammatically illustrated in Fig. 7.

The late inhibition of extensors from flexors differed from the early inhibition in being very heavy and of considerable duration (cf. MALCOLM 1955). In Fig. 8 the size of the monosynaptic potential from gastrocnemius conditioned by a supramaximal stimulation of tibialis anticus is plotted against interval between the shocks. As can be seen there is a complete inhibition lasting from 10 to 20 msec, then follows a very prolonged period of depression

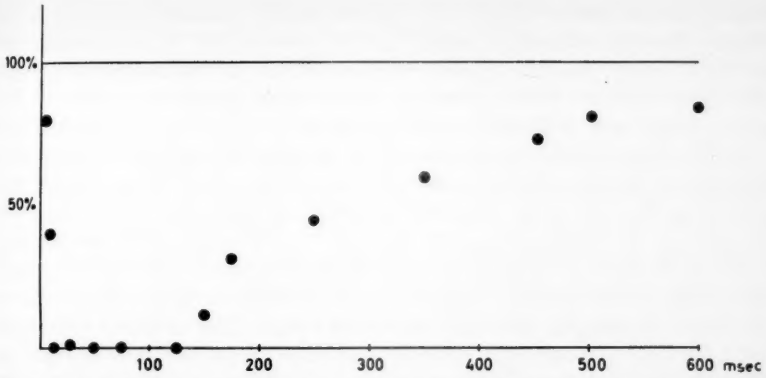

Fig. 8. 6 days old kitten. Time course of the late strong inhibition of the monosynaptic reflex from gastrocnemius set up by supramaximal conditioning stimulus to the nerve for tibialis anticus. Experimental situation as in Fig. 6. The size of the monosynaptic potential plotted in per cent of the control. Note complete inhibition from 10 up to 120 msec between conditioning and testing, and the longlasting depression. Some inhibition still left at 600 msec.

sometimes lasting up to 800 msec. This longlasting inhibition diminishes with increasing age postnatally and its duration is about 300 msec around 30 days postnatally.

Discussion

The experimental results presented are a first approach to several problems of postnatal development, which deserve a good deal more experimentation. An evaluation of the results encounters difficulties because there are two unknown factors, namely the functional activity of the dorsal root collaterals on the afferent side and of the efferent side including the ventral horn cells and their recurrent collaterals. It can, however, be stated that the afferent terminals in the newborn animal differ functionally from those of the adult animal. The missing early facilitation after a maximal homosynaptic conditioning in the newborn and young animal is a result well in accordance with the electrical properties of immature nerve fibres as originally demonstrated by HURSH (1939) on cutaneous nerves and confirmed on muscle nerves by SKOGLUND (1960 a). In the latter paper attention was drawn to the long relative refractory period in the peripheral nerve, amounting to more than 10 msec. This is probably even longer in the dorsal root collaterals and would provide an immediate explanation for the missing early facilitation. According to ECCLES (1957) the initial period of temporal facilitation passes over in 10 msec and during this period some of the less developed dorsal root collaterals might still be refractory. Furthermore, it can be stated that there is a postnatal development of the connections of the dorsal root collaterals, at least func-

tionally. It can also be stated that the efferent side is more developed functionally than the afferent to judge from the observations of the presence of an alpha rigidity and the crossed extensor reflexes in the newborn animal (SKOGLUND 1960 b). Some comments on the results presented here will be made on the basis of these general statements.

In the experiments with antidromic + recurrent activation of the motoneurones the depressant effect was found to be very small. This is partly explained by the less powerful action of recurrent activation alone. The explanation for these results might be that some recurrent collaterals are missing or do not function in the newborn animal. Another equally possible explanation is that the dorsal root collaterals which evidently develop new functional connections postnatally have not established contact with as many tonic ventral horn cells (GRANIT 1955, GRANIT *et al.* 1956) as in the adult animal. As shown by GRANIT *et al.* (1957) and KUNO (1959) it is above all these cells which have powerful recurrent inhibition. The previous results (SKOGLUND 1960 b) showed that the terminals of the spindle afferents fail to engage the tonic cells and therefore these cells may never be included in testing monosynaptically to mesure the amount of recurrent inhibition in the newborn animal. These two explanations for the development of recurrent inhibition are not at all mutually exclusive. More experimental data are, however, needed to settle this and the question of how great a part of the initial depression after maximal orthodromic conditioning is due to afterhyperpolarisation of the motoneurones and how much to less depolarising capacity of the immature afferent terminals as compared with those of the adult animal.

The state of balance between the degree of hyperpolarisation of the motoneurones and the depolarising action of the afferent terminals must also be considered when trying to explain the long and powerful inhibition of extensors from flexors and the long duration of recurrent inhibition. There may be long and powerful hyperpolarisation or less effective depolarisation, and of course any combination of these two factors is possible.

The early inhibition of flexors from extensors was found to have disappeared 10—12 msec after conditioning. This contradicts the idea that inhibition itself is of long duration. In the experimental situation however, selective stimulation of fibres mediating inhibition only is not possible (see under Methods). The late facilitation of the flexor from fibres developing into Group II fibres in the extensor might be present and would then counteract inhibition. For the missing late facilitation the reverse might be true, facilitation counteracted by inhibition. The late inhibition of extensors from flexors on the other hand presents a clear experimental situation, which can be explained with either of the two alternatives, viz. less effective depolarising action of the afferent terminals or strong longlasting hyperpolarisation of the motoneurones. The former explanation is supported by the observation that with increasing age there is found postnatal development of the afferent terminals evidenced by

1) the appearance of an early facilitation after maximal homosynaptic conditioning, 2) the appearance of early facilitation between synergists and 3) the increasing early inhibition between antagonists. It must, however, be stated that the inhibitory polysynaptic connections in the cord seem better developed than the facilitatory ones in the early stages. This difference between excitatory and inhibitory connections is further substantiated by the observation made by MALCOLM (1955) that the first signs of an afterdischarge appears 10—12 days postnatally. These aspects of the transmission through the cord will be taken up in later works.

The time at which facilitation has been found to appear postnatally is quite in accordance with the results of MALCOLM (1955). With regard to inhibition this author stated that it was not present at birth but did appear somewhat later. This has not been wholly confirmed in this investigation. The difference can to some extent be explained by the great variations in maturity between kittens even of the same litter (SKOGLUND 1960 a, b and c).

The small and unimportant inhibition of flexors from extensors in contrast to the very powerful inhibition of extensors from flexors is well in line with the predominant activity of the flexors in the newborn animal, as described in an earlier publication (SKOGLUND 1960 b). The lack of Group II facilitation of the flexors is fully compensated for by the enormous inhibition of the extensors. The change from this predominance of the flexors will probably come about by a combination of three postnatally appearing mechanisms. Firstly, a postnatal development of supraspinal inhibition of the interneurones which mediate inhibition of extensors (ECCLES and LUNDBERG 1958) has to be considered. Such supraspinal influences seem to be lacking in the newborn animal (SKOGLUND 1960 b). Secondly a diminution of inhibition owing either to the development of a stronger depolarising action of the afferent terminals or a reduction of the inhibition in itself might account for part of the development. Thirdly, as suggested by SKOGLUND (1960 b) a postnatal development of the discharge from muscle receptors, which has been shown to occur (SKOGLUND 1960 a) will rebalance flexors against extensors.

Actually in some experiments not taken up here it was found impossible to inhibit a monosynaptic extensor potential by constant stretch of an antagonist flexor muscle until 10—12 days after birth although an early inhibition could be demonstrated with electrical stimulation. This is of course partly explained by the slow development of the activity of the muscle receptors (SKOGLUND 1960 a). As will be shown, however, in a paper to follow, a postnatal development of the properties of the afferent terminals takes place which might be of importance for the mediation of tonic effects. Probably this also includes a development of the connections from the afferent terminals to tonic ventral horn cells as indicated by the observations made here on recurrent inhibition.

Summary

The postnatal development of the central connections and functions of some muscle nerves in the hind limb have been studied by means of the two-neuron arc in kittens from birth to 45 days of age.

1. Orthodromic homosynaptic conditioning at shock strength maximal for the monosynaptic potential in a newborn kitten leads to a more or less complete unresponsiveness to subsequent testing during the first 100 msec. Then follows a period of slow recovery lasting up to seven seconds. This finding points to properties of the afferent terminals different from those in the adult animal.

2. Orthodromic homosynaptic conditioning at shock strength just subthreshold for a recordable monosynaptic reflex in a newborn kitten gives a short early facilitation and a later depression of a subsequently elicited monosynaptic reflex. From this is concluded that the largest fibres are more developed than slightly thinner ones and that there is a considerable subliminal fringe.

3. Antidromic activation of the motoneurones in a newborn kitten gives a smaller depression of the monosynaptic reflex than in the adult animal. This is partly explained by a less strong effect of the simultaneously elicited recurrent inhibition. While antidromic activation does not depress motoneurone transmission for longer time than in the adult animal, pure recurrent inhibition is found to have a longer duration than in the adult stage.

4. It is found that facilitation between synergists does not appear until some time after birth. From this is concluded that there is a postnatal development of the dorsal root collaterals.

5. A small early inhibition of flexors from extensors has been found in the newborn kitten, which is stronger than the early inhibition of extensors from flexors. From this is also concluded that there is a postnatal development of the dorsal root collaterals.

6. The late inhibition of extensors from flexors is extremely potent and of long duration, which contrasts to the lacking late facilitation of flexors by extensors, and of flexors by flexors. These findings indicate that the inhibitory mechanisms are relatively better developed than the facilitatory ones in the newborn animal.

7. The strong and longlasting inhibition of the extensors from flexors provides good explanation for the predominant flexor activity described in earlier papers which are discussed in the light of the results presented.

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The Reactions to Tetanic Stimulation of the Two-Neuron Arc in the Kitten

By

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Abstract

SKOGLUND, S. *The reactions to tetanic stimulation of the two-neuron arc in the kitten.* Acta physiol. scand. 1960. *50.* 238—253. — The postnatal development of the post-tetanic potentiation of the two-neuron arc is studied in kittens ranging in age from 1 to 45 days. It is shown that post-tetanic potentiation is lacking in the newborn animal and develops with increasing age of the kittens. Evidence is presented that the postnatal development of this mechanism is running a parallel course with the maturation of the afferents and the formation of new functional connections to the motoneurones. It is concluded that post-tetanic potentiation plays an essential role for the appearance of the tonic stretch reflex and that it together with simultaneously developing neural mechanisms, earlier studied, contributes to the maintenance of posture and locomotion.

By post-tetanic potentiation of the monosynaptic reflex, as described by LLOYD (1949), the number of ventral horn cells responding is increased by mobilization of available fringe neurones. In an important paper GRANIT (1956) showed that post-tetanic potentiation acted as temporal summation in that brief tetani separated by pauses summed their effects. This is however not the only effect set up in a monosynaptic path after it has been subjected to tetanic stimulation. By recording from single fibres in the ventral root GRANIT (1955) could also show that temporal summation set up either by repeated pulls on a muscle or tetanic stimulation of a muscle nerve caused some motoneurones to fire long trains of impulses in response to a subsequent extension of the muscle. GRANIT *et al.* (1956) using the same method found

that the ventral horn cells could be divided in two categories called phasic and tonic with respect to the muscle afferents. In a later publication by GRANIT *et al.* (1957) it was shown that the ventral horn cells could also be differentiated in the same way by pinna and crossed extensor reflexes. In these cases tonic interneurones had to be postulated. The mechanism by which some ventral horn cells are caused to fire long trains of impulses must be a very powerful one forcing their muscles to tonic contractions. The muscle spindles under the control of the gamma efferents will be able to set up frequencies of discharge high enough to induce potentiating effects of this type. This mechanism must certainly be of very great importance for the elicitation and maintenance of tonic stretch reflexes.

In earlier experiments on the postnatal development of decerebrate rigidity and tonic stretch reflexes in kittens (SKOGLUND 1960 a) it was shown that alpha rigidity is present already at birth while gamma rigidity and tonic stretch reflexes develop later, appearing first in the forelimbs a fortnight after birth and after three weeks in the hindlimbs. The explanation for the missing tonic stretch reflexes in the newborn kitten was provided in a later publication (SKOGLUND 1960 b) where it was shown that the muscle spindles in the newborn and young animal only respond phasically to stretch. With increasing age of the kitten the muscle spindles begin to discharge repetitively in response to stretch and later on they can be activated by the gamma efferents. The postnatal development of the tonic discharge from the muscle spindles provides a good explanation for the late appearance of tonic stretch reflexes but in a preceding paper (SKOGLUND 1960 c) it was shown that the central terminals of the muscle afferents in a newborn kitten also have properties different from those of an adult animal. A delayed development of the central connections and functions of the muscle afferents might therefore play a role for the appearance of tonic stretch reflexes. It will here be shown that the terminals of the muscles afferents in the newborn and young kitten have properties that even in the presence of a fully developed function of the muscle spindles would prevent the creation of such longlasting facilitatory effects as seem necessary for the elicitation of tonic stretch reflexes (GRANIT 1955). Post-tetanic potentiation will be shown to be lacking in the newborn kitten and to appear some time after birth.

Material and Methods

The experiments were performed on kittens ranging in age from 1 to 45 days. A great deal of the animals belong to the material presented in a preceding paper (SKOGLUND 1960 c). Most of the animals used were anesthetized with Nembutal, 20—30 mg/kg body weight. The experimental findings have, however, been confirmed in decerebrated, unanesthetized preparations. The only difference seen between the two types of preparation with regard to the experimental findings presented here, was that the post-tetanic potentiation obtained in the older animals was usually of shorter duration and lesser magnitude in the decerebrated than in the anesthetized preparation.

The experimental arrangements were in general like those in a preceding paper (SKOGLUND 1960 c). The only new equipment used was a tetanus unit triggering the square wave stimulator at any desired frequency from 1 to 800/sec. Tetanization was always performed with shock strength well supramaximal for the nerves used. Tetanization of the immature nerves introduces some difficulties, however, which must be considered here because they are of great importance for the interpretation of some of the experimental results. As shown in an earlier publication (SKOGLUND 1960 b) the relative refractory period of the muscle afferents when the fastest fibres conduct at 10 m/sec is more than 10 msec. This means that at high frequencies of afferent stimulation some fibres are blocked at the site of stimulation. Usually a shock 3 times suprathreshold strength was used. Recording the afferent volley showed that there was quite a variation in its size at higher frequencies. Amongst the fibres becoming refractory there might quite well be those that contribute to the monosynaptic response. This means that their terminals will be unaffected by the afferent tetanization. On the other hand, when testing is performed all fibres are certainly activated, but the alterations set up in the monosynaptic pathway will be dependent on stimulus frequency, duration of tetanus and degree of maturation of the various fibres. This question will be taken up in the Discussion.

Usually the nerves to gastrocnemius and tibialis anticus were used and the monosynaptic response recorded from the cut ventral roots L 7 and S 1. The nerves to quadriceps and biceps-semitendinosus were, however, also used as well as the monosynaptic reflex recorded from the cut ventral root L 6.

Results

The effects of homosynaptic tetanization. The effects obtained in a monosynaptic pathway of a newborn kitten following tetanization of the afferents are shown in Fig. 1. The first record (c) is the control response, then follows a 12 sec tetanus at a frequency of 470/sec, indicated by the second record. In the subsequent testing of the monosynaptic potential at 3 sec intervals is seen that no response appears until 6 sec after the tetanus. Furthermore, the latency of this response is increased as much as 3 msec. In the following records the size of the monosynaptic potential gradually increases and the latency shortens so that the potential has regained its control size after 27 sec, and its control latency some 10 sec later. Thus no powerful potentiation of the monosynaptic reflex described by so many authors from LLOYD (1949) onwards is present in the newborn kitten.

No trials have been made to determine the exact time at which the first response after tetanization appears. From the experiment illustrated in Fig. 1 it is however quite obvious that the period of unresponsiveness following tetanization is considerably longer in the newborn animal than in the adult, as determined for the latter by ECCLES and RALL (1951 a). Furthermore, each shock is not followed by a small monosynaptic potential as has been found to be the case in the adult animal (HAGBARTH and NAESS 1951, ECCLES and RALL 1951 b). This is also in accordance with the findings presented in a preceding paper (SKOGLUND 1960 c) according to which there is no early

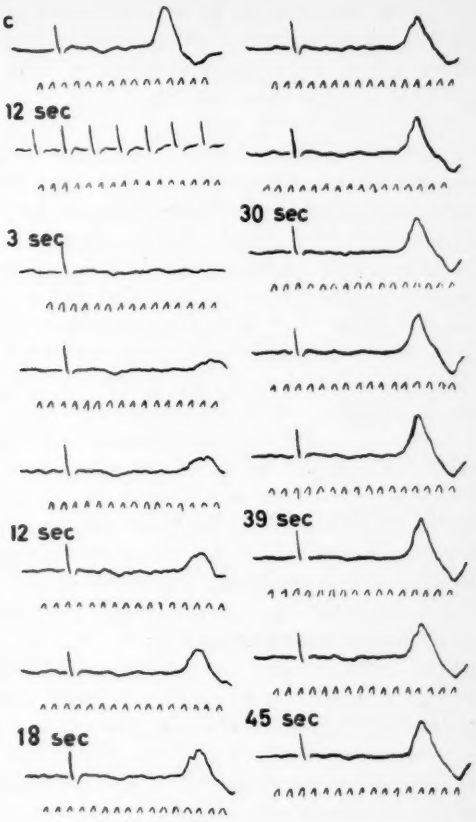

Fig. 1. One day old kitten, anesthetized with Nembutal. In c monosynaptic reflex recorded on cut ventral root S 1 on supramaximal stimulation of the nerves to gastrocnemius. All ventral roots cut and all dorsal roots from L 2 downwards except L 7 and S 1. In the second record the nerves to gastrocnemius are tetanized at a frequency of 470/sec. Duration of tetanus 12 sec, only one sweep shown here. In the following records the monosynaptic reflex is tested every third second. Time 1,000 c/sec. For further explanations see text.

facilitation of the monosynaptic reflex and sometimes no response whatever following homosynaptic conditioning at shock strength maximal for the reflex.

The first signs signalling postnatal appearance of post-tetanic potentiation is that the monosynaptic reflex does not disappear after a tetanization of short duration and that the increase of latency is very small. As seen in Fig. 2 A, where the dorsal root S 1 is tetanized with 470 shocks/sec for 3 sec, the reflex response appears only somewhat reduced 3 sec after the tetanus and the latency-increase is only about 1 msec. The monosynaptic potential quickly recovers in the following records. In the series of records shown in B, which is in direct continuation of the last record in A, a new tetanization at the same frequency and duration as in A was applied. It is seen that the events taking place in A are repeated in B. Then in C, which is in direct continuation with

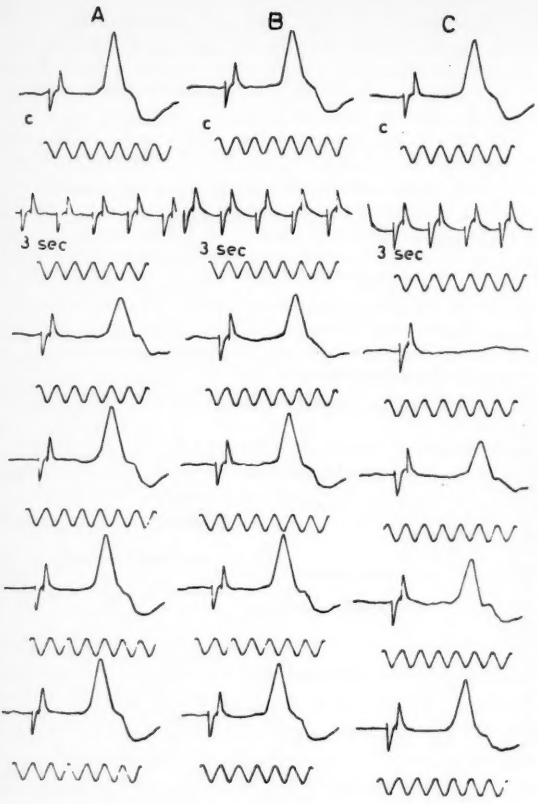

Fig. 2. 10 days old kitten, anesthetized with Nembutal. Monosynaptic reflex recorded from the nerves to gastrocnemius on stimulation of dorsal root L 7. All ventral roots cut and all dorsal roots except L 7. In A, c, is seen the control response, then follows a 3 sec tetanus applied to the dorsal root L 7 at frequency 470/sec and in the following records the monosynaptic reflex is tested at 3 sec intervals. In B, which is in direct continuation of A the same procedure is repeated as in A after the control c. C is in its turn in direct continuation with the last record in B. Time 1,000 c/sec. For further comments see text.

the last record in B, the tetanus is repeated again. Now the response has disappeared in the first record 3 sec later but from the second record after tetanization there is only a repetition of what happened in A and B. Thus, at this stage of development the monosynaptic reflex stands repeated tetanization quite well but no post-tetanic potentiation appears.

In a preceding paper (SKOGLUND 1960 c) evidence was presented that some of the largest fibres, to judge from the early facilitation obtained after homosynaptic conditioning at shock strength subthreshold for a recordable monosynaptic reflex, are more mature than the majority of the fibres contributing to the reflex. This led to the question whether selective tetanization of the largest fibres would give a post-tetanic potentiation earlier after birth than when using the full monosynaptic potential available. In testing the whole monosynaptic potential after tetanization, small effects of potentiation might

be concealed in the sum total of events. In view of the "threshold play" and the small effects that might be expected, testing at a submaximal shock strength for the monosynaptic reflex after tetanization with supramaximal shocks proved not a reliable enough method, and the variations in the control size of the potential were too big to allow any evaluation of the effects obtained.

It is probably not necessary to emphasize that it is only the testing that can be made at a shock strength not supramaximal for the monosynaptic reflex while tetanization always has to be performed with very strong shocks. By using too weak stimuli for tetanization, in the beginning of this investigation, effects resembling post-tetanic potentiation were found when the testing intervals were too short for full recovery of the monosynaptic reflex (cf. SKOGLUND 1960 c). However, some muscle afferents proved to be blocked at the site of stimulation (see Methods) giving their afferent terminals in the spinal cord a chance to recover during the tetanization period and when tested afterwards the reflex potential was larger than the control and diminished with each testing thereby imitating post-tetanic potentiation.

The methods used to study the first appearance of post-tetanic potentiation of the more mature fibres has been to elicit the monosynaptic reflex with very short intervals so that only the most mature fibres will be tested (cf. SKOGLUND 1960 c). Eliciting the monosynaptic reflex with 1.0 to 1.5 sec interval will give a control that is only 50 % of the full monosynaptic potential in the newborn kitten, as shown by SKOGLUND (1960 c). Tetanizing the muscle nerve at shock strength supramaximal for 100 % reflex potential and testing afterwards with short intervals offers the opportunity to study the effect on the most mature fibres only. It was soon found that a small post-tetanic potentiation could be obtained very much earlier postnatally with this method of testing than when using the monosynaptic response of all fibres.

In applying the above outlined method it was found that a small post-tetanic potentiation could be obtained 10—12 days postnatally. Postnatal time, as pointed out in earlier publications (SKOGLUND 1960 a and d), is, however, not a good measure of neural maturation. Therefore it was decided to correlate the appearance of post-tetanic potentiation with the conduction velocity of the fastest afferents in the muscle nerve. It was then found that a small post-tetanic potentiation invariably was obtained when using short test intervals at the time when the conduction velocity of the fastest afferents had reached 20 m/sec. The appearance of potentiation was, however, also depending on the frequency and duration of the applied tetanus.

LLOYD (1949) showed that the reactions in a monosynaptic pathway following tetanization vary with the rate and duration of the applied tetanus. He also demonstrated a definite potentiation of the monosynaptic reflex after a stimulation at frequency 15.5/sec, whereas the maximum effect was obtained at rate 300/sec. In Fig. 3 is shown the effect of varying afferent stimulus on the reaction of the monosynaptic reflex in a 12 days old kitten, where the

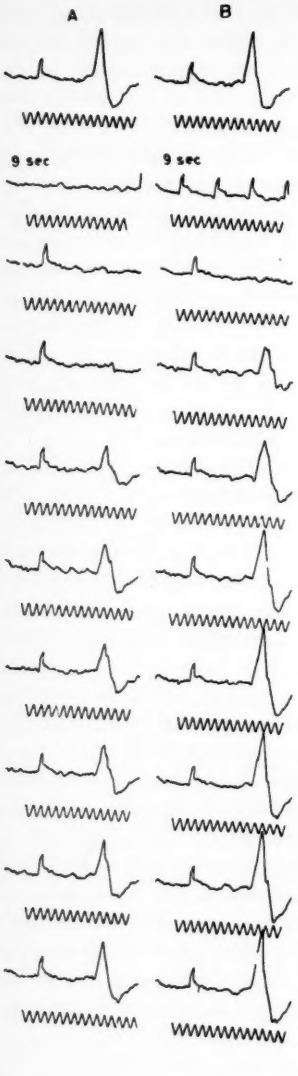

Fig. 3. 12 days old kitten, anesthetized with Nembutal. Monosynaptic reflex recorded on cut ventral root L 7 and stimulation of the nerve to tibialis anticus. All ventral roots cut and all dorsal from L 2 and downwards except L 7. In A is first seen the control response, then follows a 9 sec tetanization of the muscle nerve at a frequency of 15/sec, indicated in the second record. After tetanization the monosynaptic reflex is tested at 2 sec intervals. In B the same procedure is repeated but now the tetanus frequency is increased to 220/sec indicated by the second record. Then follows the testing of the monosynaptic response and a small post-tetanic potentiation can be seen. Time 1,000/sec. For further comments see text.

conduction velocity of the fastest muscle afferents from the gastrocnemius was 21 m/sec. The reflex was tested at an interval of 1.5 sec. In A is first seen the control response then follows a tetanus at frequency 15/sec for 9 sec. In the subsequent records there is seen a long depression and no potentiation. In B

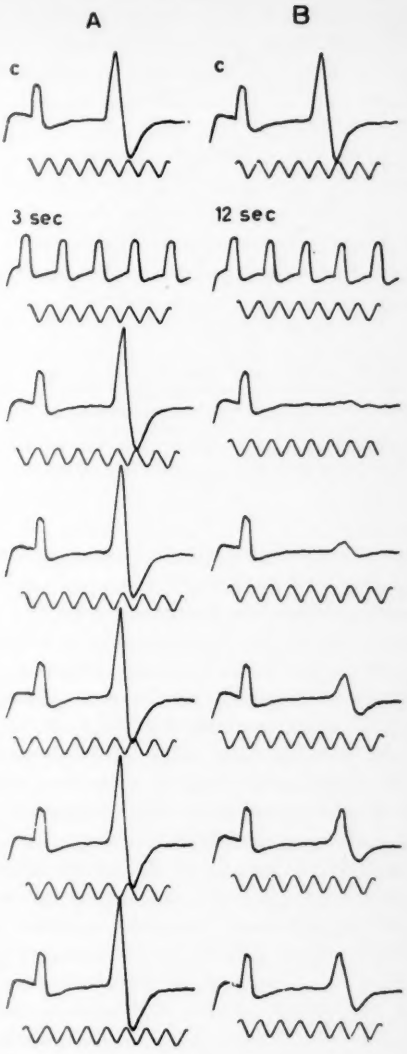

Fig. 4. 14 days old kitten, anesthetized with Nembutal. Monosynaptic reflex recorded on cut ventral root S 1 on stimulation of the nerves to gastrocnemius. Conduction velocity of the fastest afferents 26 m/sec. In A is first seen the control (c), then follows a tetanization of the muscle nerve for 3 sec at a frequency of 500/sec, indicated by the shock artefacts in the second record. Then follows testing of the monosynaptic reflex after tetanization of 2 sec intervals. In B the same procedure is repeated but the muscle nerve is tetanized for 12 sec, as indicated in the second record. Time 1,000 c/sec. For further comments see text.

on the other hand the control in the uppermost record is followed by a tetanus at frequency 200/sec and the initial depression is succeeded by a small post-tetanic potentiation. The experimental conditions are given in the legend.

In his experiments on the effect of varying duration of the tetanus LLOYD

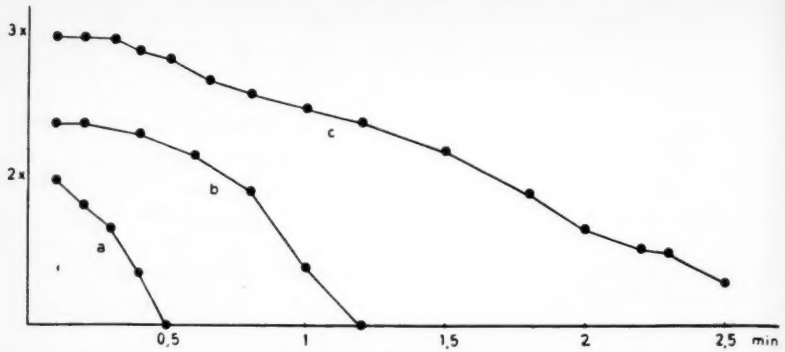

Fig. 5. Intensity and duration of post-tetanic potentiation in 3 different animals of different age. In all three experiments the monosynaptic potential recorded from the cut ventral root S 1 on stimulation of the gastrocnemius nerves was used. The muscle nerve was tetanized 6 sec at a frequency of 315/sec. The first value is taken 6 sec after the end of the tetanus, and plotted in multiples of the control response on the ordinate against time in minutes on the abscissa. Curve a is from a 14 days old kitten, b from a 23 days old kitten and curve c from a 28 days old kitten.

(1949) found that a ceiling was reached with a duration of 10 sec. Once the ceiling was reached a further prolongation of the tetanus only resulted in a more prolongated potentiation. In Fig. 4 is seen the effect of varying tetanus duration for the appearance of a post-tetanic potentiation in a 14 days old kitten. In A is first seen the control, then follows a 3 sec tetanus at a rate of 500/sec. A small potentiation is obtained when the monosynaptic reflex is tested at an interval of 2 sec. In B, on the other hand, the duration of the tetanus is increased to 12 sec but kept at the same frequency as in A. Now a depression follows which is not converted into any post-tetanic potentiation.

In the experiments illustrated in Fig. 3 and 4, when using short test intervals, a small post-tetanic potentiation could thus be demonstrated when the conduction velocity of the fastest afferents had reached 20 m/sec. No investigations have been made to uncover any early potentiation after very short tetani, like the one demonstrated by ECCLES and RALL (1951 a) in the adult animal. When the monosynaptic response was tested at longer intervals from 3 to 5 sec, potentiation did not occur until the fastest fibres had reached a conduction velocity of 30 m/sec unless very high frequencies of stimulation were used. This will be further discussed later on. At this stage of development the whole monosynaptic reflex was tested at a shock interval of 3—4 sec, as described in an earlier publication (SKOGLUND 1960 c). The potentiating effect then obtained was variable and increased with further increase of the conduction velocity, as shown in Fig. 5, where the amount of potentiation and its duration is plotted for three different stages of development. At a conduction velocity of 30 m/sec brief tetani are also summed (cf. GRANIT 1956).

Potentiation of inhibition. It might be argued that part of the long depression seen after homosynaptic tetanization of a muscle nerve, is brought about by tetanization of fibres with autogenetic inhibitory connections, as inhibition has sometimes been found to be more effective than facilitation in the earlier stages of development (SKOGLUND 1960 c). The action of Group II and III fibres might also play a role, but as no difference has been seen between flexors and extensors the effect of these high threshold fibres can hardly be significant (cf. ECCLES and RALL 1951 b). With regard to Group I fibres with autogenetic inhibitory actions, their effect is probably not fully developed in the newborn and young kitten as suggested by the weak early inhibition seen between antagonists (SKOGLUND 1960 c). However, it is of interest to study the effect of tetanization on inhibition.

In Fig. 6 is illustrated the effect of tetanization of the nerve to tibialis anticus on the monosynaptic reflex from gastrocnemius. In A is first seen the unconditioned monosynaptic response from gastrocnemius recorded on the cut ventral root L 7. In B and C this reflex potential is inhibited by conditioning the nerve to tibialis anticus whose monosynaptic reflex is also in the same record. In the records from D onwards the nerve to tibialis anticus is tetanized at a frequency of 250/sec with shocks supramaximal for it. As can be seen the inhibition of the gastrocnemius monosynaptic potential diminishes successively during tetanization of the antagonist in the records from D to G (cf. HAGBARTH and NAESS 1951), by comparison with the control with single shocks to tibialis anticus (B and C). This reduction of inhibition during tetanization of tibialis anticus is simply due to blocking of tibialis anticus fibres. Then in H conditioning with one shock is seen to exert very little inhibition three seconds after the end of tetanization. The inhibitory effect increases in the following records I and J, 6 and 9 sec respectively after tetanization but inhibition is not as great as in B and C. It can further be noticed that the monosynaptic reflex from tibialis anticus is absent in H and reappears in I with increased latency. From the experiments illustrated in Fig. 6 it is thus apparent that there is no post-tetanic potentiation of inhibition in the newborn kitten as in the adult animal (cf. LLOYD 1949). After this experiment the possibility that part of the depression after homosynaptic tetanization was caused by potentiation of autogenetic inhibitory fibres also appears less likely.

Discussion

LLOYD (1949) in his description of the phenomenon of post-tetanic potentiation pointed out that following tetanic stimulation of the afferent fibres there follows a period characterized by a prolonged positive afterpotential during which the intramedullary collaterals are hyperpolarized. This hyperpolarization leads to an increase of the intramedullary spike (LLOYD 1949, ECCLES and RALL 1951 a). This is one very potent factor in the post-tetanic

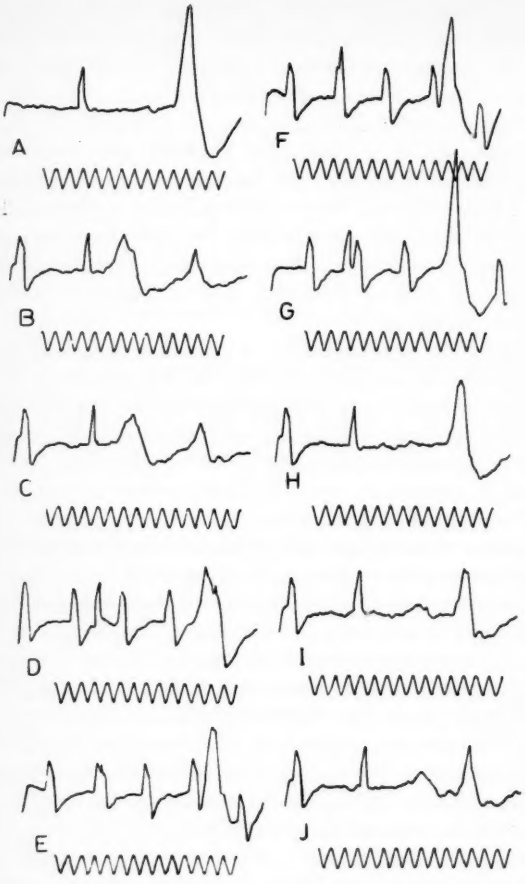

Fig. 6. 9 days old kitten, anesthetized with Nembutal. In A is seen the monosynaptic reflex recorded on the ventral root L 7 on stimulation of the nerves to gastrocnemius. In B and C the monosynaptic reflex from gastrocnemius is inhibited by stimulation of the nerve to tibialis anticus, which also itself sets up a monosynaptic reflex in the ventral root L 7. In the records B and C are seen from left to right the shock artefact on stimulating tibialis anticus, then the shock artefact from gastrocnemius closely followed by the monosynaptic reflex from tib. ant. and at last the inhibited monosynaptic reflex from gastrocnemius. All ventral roots cut and all dorsal from L 2 downwards except L 7 and S 1. In the records from D to G the nerve to tib. ant. was tetanized at frequency of 215/sec while still eliciting the monosynaptic reflex from gastrocnemius and it can be seen that the inhibition diminishes. Then in H 3 sec after tetanization the same procedure as in B and C is repeated and it can be seen that inhibition is much smaller than before tetanization and increases in the following records I and J, 6 and 9 sec after tetanization respectively. Time 1,000 c/sec. The increase of the monosynaptic response in G above the control in A is caused by one of the shock artefacts coinciding with the peak of the response.

potentiation of the monosynaptic reflex (cf. ECCLES and KRNJEVIĆ 1959). WALL and JOHNSON (1958), in reinvestigating the post-tetanic potentiation of the monosynaptic reflex arc, found, by microelectrode stimulation, that the excitability of the primary afferent fibres was decreased during tetanization while the motoneurones were unaffected. These authors also concluded that potentiation is probably attributable to hyperpolarization of the presynaptic fibres. They suggested that the delayed onset of potentiation, varying with frequency

and duration of the tetanus is attributable to presynaptic events, either an anodal block or desynchronization owing to decrease in conduction velocity in the hyperpolarized terminals.

The findings in the newborn kitten, presented here, seem to support the propositions of WALL and JOHNSON completely. It thus appears as if the events seen in Fig. 1 are but an exaggeration of those taking place in the adult animal. Firstly anodal block causes complete disappearance of the monosynaptic reflex, secondly when the reflex potential reappears it has an increased latency on account of decreased conduction velocity in the hyperpolarized fibres with a desynchronization of the incoming volley as a consequence. A decrease of the monosynaptic potential should thus be a consequence of the combined effects of anodal block and desynchronization.

Hyperpolarization of the terminals is here accepted as the immediate cause of the events appearing after tetanization in the newborn animal. LLOYD's original statement (1949) was that potentiation is a function of hyperpolarization which in its turn is a function of the frequency and duration of the applied tetanus, increasing with both these factors up to certain limits. From this should follow that increase of hyperpolarization leads to an increase of potentiation unless anodal block occurs. The depression of the monosynaptic response in the newborn animal is explained by hyperpolarization. The depth and duration of depression is consequently a function of hyperpolarization too and likely to vary with the same factors namely frequency and duration of the tetanus.

In the experiment illustrated in Fig. 3, however, a tetanus of low frequency is seen to cause a long depression and no potentiation while a tetanus of higher frequency is seen to give a shorter depression and a small potentiation. This seems to contradict the earlier statements. The explanation is readily obtained if it is recalled that a high frequency is likely to block some fibres (see Methods) at the site of stimulation thus leaving some terminals uninfluenced. With the low frequency stimulation on the other hand each volley is likely to reach the terminals leaving in its wake a considerable hyperpolarization. Thus a high frequency stimulation which blocks some of the more immature fibres at the site of stimulation will help to uncover the potentiation set up by the more mature ones. In the experiment illustrated in Fig. 4, a short tetanus is seen to give a small potentiation while a longer one at the same frequency gives depression only. This is quite in conformity with what happens in the adult animal. After tetanizations of long duration, there ensues a certain time before full potentiation is built up, a fact which can be explained by hyperpolarization blocking some fibres.

So far only the cause of the depression has been considered but why is there in the newborn animal no potentiation when the reflex response has regained control latency and size? The reply to this might be that when some fibres start to respond others are still blocked and when the latter start

to conduct the former would have passed out of their hyperpolarized state. Recalling, however, that potentiation in the adult animal according to LLOYD (1949) lasts up to 8 minutes the missing post-tetanic potentiation cannot be explained in this way unless hyperpolarization is assumed to be of shorter duration in the immature fibres. This seems very unlikely, but in fact, as shown in Fig. 5, potentiation does not only increase in magnitude with increasing age of the animal but also in duration. The most likely explanation for the appearance of post-tetanic potentiation is furnished by the observations in a preceding paper (SKOGLUND 1960 c) that there is delayed postnatal development of the functional connections of the afferent terminals. Thus because of the increasing number of neurones connected up and by maturation of the terminals post-tetanic potentiation can ultimately appear. The increase in magnitude of potentiation with increasing age is explained by 1) the faster recovery of the fibres after tetanization and 2) by the new neurones added. The increase in the duration of the potentiation also signifies that "maturation" has brought in a larger number of afferent connections of the grown-up type for the motoneurones (cf. SKOGLUND 1960 c).

The explanation for the appearance of post-tetanic potentiation put forward here focuses attention on the electrical properties of the afferents. In explaining the long depression after tetanization by hyperpolarization it must be assumed that the positive afterpotentials are more pronounced in immature fibres than in mature ones. This has also been shown to be the case (HURSH 1939).

At the stage of development when the first signs of post-tetanic potentiation appeared under certain experimental conditions, the conduction velocity of the fastest afferents had reached 20 m/sec. As described in a preceding paper (SKOGLUND 1960 b) the absolute refractory period of the afferents attain adult values when they conduct 20 m/sec. No investigation has been performed hitherto to ascertain if the positive afterpotentials of immature fibres also reach adult values at the same time.

According to SKOGLUND (1960 d) the fastest muscle afferents develop at the highest rate and as a consequence they will attain an even higher conduction velocity than 20 m/sec until the slowest fibres contributing to the monosynaptic response have attained that velocity. According to LLOYD (1941) the Group I fibres ranging from 12 to 20 microns have monosynaptic connections. If it is now assumed that the slowest fibres contributing to the monosynaptic reflex response have to reach a conduction velocity of 20 m/sec corresponding to about 3 microns, until they can contribute to post-tetanic potentiation, it is easily calculated according to SKOGLUND (1960 d) that the fastest fibres must have reached 5 microns, corresponding to a conduction velocity of 30 m/sec. By comparison with the experimental results presented here, that the whole monosynaptic reflex can be potentiated, when the fastest afferents conduct 30 m/sec these assumptions seem to hold true.

For the appearance of post-tetanic potentiation the postnatal development of new functional connections to ventral horn cells have been emphasized. In a preceding paper it was shown that recurrent inhibition, which by GRANIT *et al.* (1957) has been demonstrated to be strongest on the tonic ventral horn cells (GRANIT *et al.* 1956) was very small in the newborn kitten. One explanation given for this was that the dorsal root collaterals had made connections with very few such motoneurones. Be this as it may, as long as events such as those found here in the newborn animal take place following tetanization of the afferents, it is very unlikely that any driving of the tonic ventral horn cells from the muscle afferents could occur (cf. GRANIT *et al.* 1956), even if the muscle receptors were firing tonically (cf. SKOGLUND 1960 b). This adds new aspects to the explanation of why the tonic stretch reflex is absent in the newborn kitten (SKOGLUND 1960 a).

Summing up earlier results on the postnatal development the most conspicuous feature of immature nervous structures is the slow conduction velocity and the low excitability. The latter is exemplified by high thresholds to electrical excitation and long refractory periods. Nervous interconnections will certainly be formed early in intrauterine life, but the nervous processes will be limited by the abovementioned factors. The stretch-reflex arc attains adult conduction time when the conduction velocity reaches 30 m/sec owing to the shorter conduction distances compared with the adult animals (SKOGLUND 1960 d). This becomes significant (cf. GRANIT 1955) in the light of the observations that the muscle spindles then fire tonically and are under control of the gamma efferents (SKOGLUND 1960 b). The development of these features have been shown to run parallel with the maturation of the electrical properties of the nerve fibres just as the development of post-tetanic potentiation. Such a scheme of development has of course to be tested in other reflex arcs than the stretch reflex to become generally valid. Anyhow all the experimental evidence goes to show that the maintenance of muscular tone is achieved by the gamma system working on the muscle spindles and these in turn on the tonic ventral horn cells thereby running the tonic stretch reflex. Not until the time when all these mechanisms have developed do the animals achieve an upright position and start to walk. Then also tonic stretch reflexes and gamma rigidity can be demonstrated. The results are therefore in full accordance with the views developed by GRANIT (1957) and his collaborators on the role of the gamma system in tonic control.

Summary

The reactions of the afferent terminals of muscle nerves after tetanic stimulation are studied in the two-neuron arc of kittens ranging in age from birth to 45 days.

1. It is shown that post-tetanic potentiation of the monosynaptic response

is lacking in the newborn animal and develops with increasing age of the kittens.

2. Tetanic stimulation of the monosynaptic response in the newborn animal is followed by a complete disappearance of the response for several seconds. The reappearing response has increased latency and decreased size and returns to the control after half a minute or longer. These reactions are explained by hyperpolarization of the afferent terminals. The same effects take place when stimulating inhibitory fibres.

3. The degree of depression and/or potentiation of the monosynaptic response is varying with the frequency and duration of the tetanus. These variations can be explained by both peripheral and/or central effects of tetanization.

4. Evidence is presented that the appearance of post-tetanic potentiation is related to the maturation of the afferent terminals and the development of new functional connections to motoneurones.

5. It is found that the whole monosynaptic response can be potentiated when the fastest afferents conduct 30 m/sec. It is calculated that the slowest fibres contributing to the reflex response have reached a conduction velocity of 20 m/sec at that stage of development. From this and earlier obtained experimental results it is concluded that the afferents attain adult electrical properties when reaching a conduction velocity of 20 m/sec.

6. Post-tetanic potentiation has been found to develop simultaneously with other neural mechanisms which contribute to the stretch reflex, why it is concluded in conformity with the results from adult animals that the appearance of this mechanism contributes to the postnatal development of posture and locomotion.

This work is part of a series of investigations into the postnatal development of postural reflexes supported by the Swedish Medical Research Council.

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Circulatory and Respiratory Adaptation to Severe Muscular Work

By

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Abstract

ÅSTRAND, I., P.-O. ÅSTRAND, E. H. CHRISTENSEN and R. HEDMAN. *Circulatory and respiratory adaptation to severe muscular work.* Acta physiol. scand. 1900. *50*. 254—258. — A trained subject performed a constant work output per min (2,160 kpm/min or 360 Watts) with variations in work time from 0.5 min up to the maximum tolerable time of 9 min. The chronological changes in different functions, such as heart rate, pulmonary ventilation etc. responsible for oxygen transport were recorded. At a given time after the start of work the subject reached identical values for these functions and for O_2 uptake, independent of the total length of time which was known to the subject, when the work started. The heart rate and other related functions of importance concerning oxygen uptake are narrowly regulated by the work output and the fitness of the subject and are apparently to an exceptionally high degree resistant to changes in the mental state of the trained subject.

More detailed information is needed regarding the reaction of the human organism during severe muscular work where part of the energy has to be delivered by anaerobic processes and where an increasing concentration of lactic acid consequently will limit duration of work. In such a situation the organism will be in a state of constant change, and it is of interest to measure and to analyze the chronological changes in the different functions, such as heart rate, pulmonary ventilation etc., responsible for oxygen transport.

Table I. Mean value, error of the mean and standard deviation of heart rate, pulmonary ventilation and oxygen uptake during work of 2,160 kpm/min performed between 0.5 and 9.0 min

Work output 2,160 kpm/min	Testing time min	Blood lactate conc. mg/100 ml post-exercise values	Heart rate				$\dot{V}_E$, l BTPS				$\dot{V}_{O_2}$, l STPD			
			Mean	ε	S.D.	n	Mean	ε	S.D.	n	Mean	ε	S.D.	n
	0.0–0.5	42	121	±1.6	±5.6	12	32.7	±1.7	±7.0	17	1.45	±0.04	±0.18	17
	0.5–1.0	49	143	±1.1	±4.1	14	58.4	±0.8	±3.0	13	3.08	±0.03	±0.10	13
	1.0–1.5		154	±1.3	±4.2	10	80.4	±1.3	±4.3	10	3.62	±0.03	±0.10	10
	1.5–2.0	62	158	±1.1	±3.9	12	90.7	±1.6	±4.9	9	3.80	±0.02	±0.06	9
	2.0–3.0	86	163	±0.9	±2.9	11	98.4	±1.5	±4.3	8	3.95	±0.03	±0.08	8
	3.0–4.0	110	168	±0.8	±2.5	10	104.6	±1.7	±4.6	7	4.16	±0.01	±0.02	7
	4.0–5.0	125	173	±1.3	±3.4	7	108.2	±1.2	±3.0	7	4.31	±0.03	±0.08	7
	5.0–6.0	131	177	±1.1	±2.4	5	112.7	±1.5	±4.8	10	4.44	±0.02	±0.08	10
	6.0–7.0	144	182	–	–	2	118.1	±1.0	±2.2	5	4.50	±0.02	±0.04	5
	7.0–8.0	144	180	–	–	2	122.1	±2.7	±5.4	4	4.59	±0.02	±0.04	4
	8.0–9.0	152	186	–	–	1	130.4	–	–	2	4.65	–	–	2

If extremely heavy work has to be carried on to exhaustion, taking 5 or 10 min, the subject has to be highly motivated with all the consequences this may have for instance on the adrenal system. Thus an entirely different mental state may prevail when he knows he has only to work for 30 sec, and that might influence the results.

We have tried to attack some of the above mentioned problems simply by letting a trained subject perform a constant work output per min with variations in work time from 0.5 min up to the maximum tolerable time of 9 min. He was always informed about his daily work program before start of the experiments.

Material and Methods

All experiments were performed with the physically well trained male subject R. H. The majority of the experiments were done in 1954, complementary ones were done four years later. At the time of the first experiments, R. H. was 25 years of age, his weight was 74 kg and height was 177 cm. His maximal oxygen uptake, when working on the bicycle ergometer with a load that he could stand for 5 or 6 min, was 4.6 l per min, or 62 ml/kg/min. His basal O_2 uptake was 0.260 l/min and his basal heart rate averaged 49 beats per min. Four years later these values were unchanged. An analysis of the results from the two series of work experiments showed that even they were identical from a statistical point of view and all values are included in the results of Tab. I.

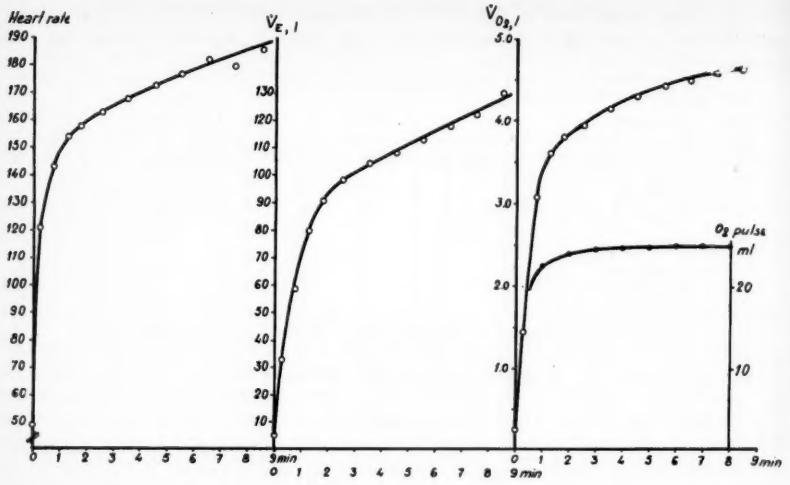

Fig. 1. Heart rate, pulmonary ventilation and O_2 uptake at different times after start of work, 2,160 kpm/min.

Basal values for heart rate and O_2 uptake were measured with the fasting subject lying on a bed close to the bicycle ergometer. The work was performed on a Krogh bicycle ergometer immediately after the rest experiment without "warming up"; 60 pedal revolutions per min and a load of 6 kg corresponded to 2,160 kpm/min or 360 W. The work times varied from 0.5 min up to 9 min. The expired air was collected in Douglas bags during the whole work period and in such a way that a detailed analysis of the respiratory functions was possible even during the first min of work. The gas analysis was made on a modified Haldane apparatus. Heart rate was recorded with an electrocardiographic pulse counter. Blood lactic acid concentration was determined in arterialized blood taken from a pre-warmed finger tip. The analyses were made according to the method by BARKER and SUMMERSON (1941) modified by STRÖM (1949).

Results

Table I gives the mean values, the standard deviations and the errors of the means for heart rate, pulmonary ventilation and oxygen uptake during work. Four or more values for a certain time period were treated statistically. For obvious reasons the largest number of determinations belongs to the first min of work. The values, which refer to the same time in the work period, show at no systematical variations in spite of the fact that they were derived from experiments of entirely different durations and were collected in two series of experiments separated by as much as four years.

Figure 1 illustrates the average increase in heart rate, pulmonary ventilation and O_2 uptake at different time intervals from the beginning of work.

The heart rate reaches 150 beats per min during the first min of work. Between the 2nd and the 9th min there is an increase from 160 to 189 or about 4 beats per min. The pulmonary ventilation shows a similar steep increase, and 72 l per min are obtained at the end of the first min. From about the 2nd min the rise is slowed and corresponds to 5.3 l/min; at the 9th min the pulmonary ventilation corresponds to 130 l/min. The oxygen uptake reaches 3.4 l/min at the end of the first min; between the 2nd and the 6th min it rises from 3.85 to 4.50 l/min or 0.16 l per min; whereas during the last 3 min of work the increase is only 0.05 l per min.

The oxygen pulse, that is ml of O_2 uptake per heart beat, is practically constant, or about 25 ml, between the 2nd and the 9th min of work; at the end of the first min a value of 22.6 ml is reached. The oxygen pulse is partly determined by the stroke volume of the heart and partly by the a.-v. O_2 difference, and these functions must apparently have reached a constant value already after 2 min of work, or an increase or decrease of the one function must have been compensated by a corresponding change in the opposite direction in the other one. The latter assumption is less likely than the former.

The blood lactic acid concentration given in Table I represents the maximal post exercise values obtained after the work experiments of a duration of 0.5 min, 1 min etc. Consequently they do not represent the actual concentrations 0.5 min or 1 min after start of work, but give undoubtedly a relatively true picture of the rising concentration of anaerobic metabolites in the blood with increasing duration of work. The maximum concentration of 152 mg per 100 ml indicates that the 9 min of work represents the limit for what can be performed even with the strongest motivated trained subject, who is used to tolerate high lactic acid concentrations.

Discussion

Apprehension may have a marked influence on heart rate and respiration at rest. It is, however, a general observation that during work the psychic influence on heart rate and respiration is more or less abolished except under extraordinary conditions, where the whole emergency reaction may come into play. The here mentioned results confirm these earlier observations. At a given time after start of work the trained subject reaches identical values for heart rate, pulmonary ventilation and oxygen uptake even though he knows, that in one experiment he has to perform the relatively easy task of one min of work, and in the other one he has to go to total exhaustion. The heart rate and related functions of importance for the oxygen uptake and transport are definitely regulated within narrow limits determined by the work output and the fitness of the subject and are apparently to an exceptionally high degree resistant to changes in the mental state of the subject. If this was not the case, the reproducibility of results from one work experiment to the next

should not be as good as generally found. Thus the day-to-day variation in heart rate reaction to muscular exercise with submaximal work loads on a bicycle ergometer is very small, at least if a subject is in a good training condition. We have experience with cross country skiers taking part in as much as 4 competitions in 8 days during an Olympic game. Test performed the day before and after a race usually gives identical results. From a psychological viewpoint competitors are usually tense before the competition but very relaxed after the final start. Changes in the heart rate response, if present, can usually be explained by effects of dehydration, infections etc. and is usually also reflected in the actual performance in the competition. The criticism, that the results of the usual bicycle ergometer or treadmill test to a high degree may depend upon the mental state of the tested subject, is obviously not valid, at least not if the subjects are trained and the test load is sufficiently high.

If we assume a mechanical efficiency of 23 per cent, which is the normal average efficiency at heavy work for this subject, an O_2 uptake of 4.75 l/min would be adequate at the load of 2,160 kpm/min. This uptake was nearly obtained during the last min of work (cf Table I). The slow rise in blood lactic acid concentration during the last 4 or 5 min of work also indicates that an increasing fraction of the total metabolism is covered by aerobic processes and less and less by anaerobic.

As mentioned above the practically constant O_2 uptake per pulse beat from the 2nd min onwards might indicate a constant stroke volume and a constant a.-v. O_2 difference for the following 7 min of work. If this is so, the increase in O_2 uptake that takes place between the 2nd and 9th min of work from 3.85 l/min to 4.65 l/min, can be "explained" entirely by a simultaneous increase in heart rate, from 160 to 186 beats per min.

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Habituation of Autonomic Response Elements under two Conditions of Alertness

By

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Abstract

SCHOLANDER, T. *Habituation of autonomic response elements under two conditions of alertness.* Acta physiol. scand. 1960. *50.* 259—268. — The habituation of the phasic responses and the prestimulus values of the pulse rate and the electrodermal activity has been studied under two different conditions of alertness. The auditory stimulus was administered with intervals varied at random according to a constant sequence. The degree of alertness was assumed to be higher when the subject was preoccupied with a special sighting device than when he was relaxed. It was found that the increased alertness tended to delay the habituation and to increase the prestimulus activation level of the variables.

It has been stated by several authors (SHARPLESS and JASPER 1956, HERNANDEZ-PEON, JOUVET and SCHERRER 1957, HAGBARTH and KUGELBERG 1958, GLASER 1958) that mental processes such as attention, expectancy and apprehension may influence the course of a habituation process.

In a recent investigation (SCHOLANDER 1960) a study was made of the variability in the habituation[1] of different autonomic response elements to monotonously repeated auditory stimulation during iterated experimental sessions. Contrary to the results obtained in similar investigations made by SEWARD and SEWARD (1934) and DAVIS (1934), no tendency towards habituation between sessions was found and even within sessions this tendency was usually rather inconstant. Instead, an evident intra-individual constancy was found throughout the experimental period. It was suggested that this discrepancy from earlier results might be due to certain circumstances in the

[1] Habituation has been defined as "whatever decrement in different response elements brought about by monotonously repeated, unconditioned stimuli at sufficiently long intervals to allow complete subsidence of each acute response" (SCHOLANDER, 1961).

experimental conditions. One of the variables, viz. the pupillary reaction was recorded by means of a special camera (DUREMAN, SCHOLANDER and SÄLDE 1959). To guarantee optimal quality of the recordings the subjects were instructed to maintain a proper position in relation to this camera throughout each session. They could do this themselves by means of a simple sighting device and a small, red twinkling bulb which served as fixation point. It was suggested that the constant preoccupation with this device might result in an increased alertness which could somehow interact with the habituation process.

The aim of the present investigation was to test the correctness of this assumption. The prestimulus levels and phasic response amplitudes of the electrodermal activity (EDA) and the pulse rate were studied during repeated auditory stimulation. Intra-individual comparisons were made between the results obtained with and without the use of the sighting device.

It was predicted that the increased alertness brought about by the preoccupation with the sighting device of the pupillary camera would 1) delay the habituation process and 2) increase the prestimulus levels of the variables involved.

Methods

The laboratory and the apparatus as well as the technique of recording used in the present study have been described in detail elsewhere (DUREMAN 1959; SCHOLANDER 1960). An a. c. apparatus was used to record the EDA. The values of the skin resistance were transformed into conductance.[1] The pulse rate was recorded by means of a special level-writing device which independently of the pulse amplitude gave a direct, continuous curve showing the variations in the frequency per minute. The prestimulus level was chosen as an approximate measure of the tonic activation or tonicity level. The phasic reactivity was expressed in terms of both response amplitude, *i. e.* maximal post-stimulus change of the variables and frequency of positive reactions[2] during successive groups of five trials.

The stimulus was a "white noise" signal with a duration of 0.1 sec and an intensity of 105 db above threshold. It was administered binaurally through headphones.

The 10 subjects, 8 females and 2 males aged 23—34 years were recruited from the staff of the laboratory. Each subject served for 15 min on each of two sessions separated by an interval of 3 days. To control as far as possible diurnal variations all subjects went back at approximately the same time of the day. Furthermore they were instructed to make no deviations whatever from their ordinary routine of living.

The subjects were divided at random into two groups of equal size. To eliminate the possible transfer of habituation between sessions the experiments were undertaken in a reverse order in these groups. The subjects were informed only that the investigation was centered around the study of habituation of autonomic response elements to monotonously repeated auditory stimulation. Before one experiment they were instructed to relax, but keep their eyes open. Before the other session they were told to fix their eyes steadily upon the small, red twinkling bulb of the pupillary camera and

[1] Conductance is the inverse of the resistance (micromhos).

[2] Every deflection of the skin resistance occurring within a latency period of 1.5 to 3.0 sec. and every increase of the pulse rate occurring within a latency period of 0.5 to 3.0 sec was regarded as "positive reactions".

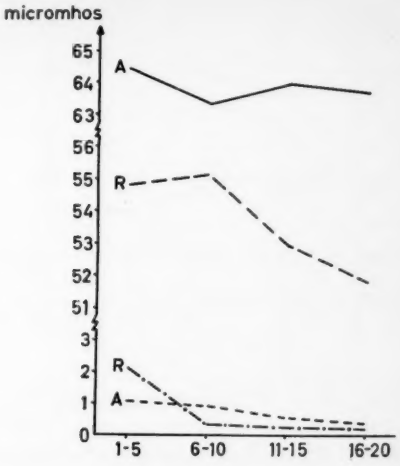

Fig. 1. The mean values of the prestimulus EDA (upper curves) and the EDR amplitude (lower curves) during successive groups of five trials during the "relaxed state" (R) and the "attentive state" (A).

be careful to maintain exactly the same position throughout the experiment. Moreover they were informed about how to control their position by means of a simple sighting device.

Except for these instructions the procedure during each session was exactly the same. After a period of 5 min during which the subject could relax and get accustomed to the apparatus, 20 white noise stimuli were administered with intervals varied at random between 20 and 40 sec. The sequence of these intervals was kept constant.

All comparisons made in the present study were intra-individual. To simplify the treatment of data and to increase the reliability of the measurements average values of successive blocks of 5 trials were computed. For comparisons between sessions the mean values of all trials were used. As a gross measure of the change of a parameter occurring within a session a so called "change index" was computed. Thus the mean prestimulus value or the mean phasic response amplitude during the last 15 trials was expressed in per cent of the average value during the first 5 trials, and the value obtained was subtracted from 100 (cf. SCHOLANDER 1960).

Non-parametric statistical methods were used in the analysis of the data. To test possible changes occurring within sessions Friedman two-way analyses of variance by ranks (SIEGEL 1956, p. 166) were used. Wilcoxon matched-pairs signed-ranks tests (ibid., p. 75) were applied to the differences between sessions. One-tailed tests of significance were used whenever the results indicated the direction of the predicted difference. As a rule the null-hypothesis (H_0) was rejected in favour of the alternative hypothesis (H_1) if the probability yielded by the above mentioned tests was equal to or less than $\alpha = 0.05$.

Results

Survey of graphs

In Fig. 1 and 2, the upper curves represent the mean prestimulus activation levels of all subjects during each session and the lower curves the corresponding mean phasic response amplitudes. The "attentive state" which implies pre-

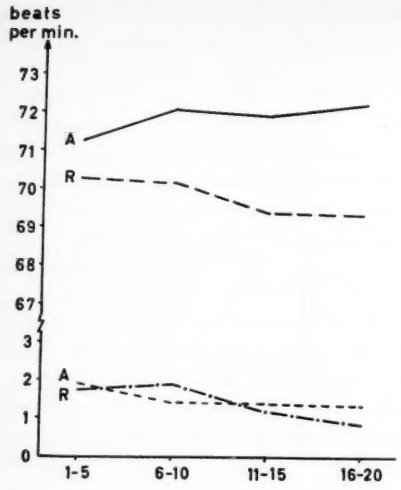

Fig. 2. The mean values of the prestimulus pulse rate (upper curves) and the pulse rate response amplitude (lower curves) during successive groups of five trials during the "relaxed state" (R) and the "attentive state" (A).

occupation with the sighting device was called A, and the "relaxed state" R. Successive groups of 5 trials have been plotted along the base line with mean skin conductance or conductance change (in micromhos) and mean pulse rate per minute or rate change during each trial-group along the vertical axis.

EDA data: A closer examination of the curves of the EDA (Fig. 1) reveals certain striking differences between the two experimental conditions. Thus the level of the prestimulus EDA is considerably higher during the "attentive state" (A) than during the "relaxed" (R). Even the slope of the curves is quite different. Curve A shows only a slight declining trend whereas curve R declines rapidly after a certain rise during the first ten trials.

As to the amplitudes of the EDR illustrated in the lower curves, the difference between the average levels seems to be rather insignificant due to the high initial value in curve R. A comparison of the slopes shows that curve A declines slowly whereas curve R falls rapidly from a rather high initial value to a very low level which keeps relatively constant during the last fifteen trials.

Pulse data: The corresponding curves of the pulse rate in Fig. 2 show a similar trend. Even here the prestimulus level is higher during the "attentive" than during the "relaxed state". Furthermore, there is a marked discrepancy in the course of these curves. Whereas curve A rises successively, curve R shows a clearcut declining trend.

The differences between the two conditions seem to be less pronounced for the pulse rate response amplitudes. The average levels of the curves are here approximately the same. There is a somewhat greater decline in curve R.

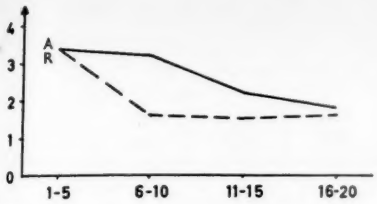

Fig. 3.

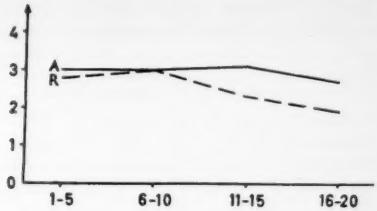

Fig. 4.

Fig. 3. The mean frequency of positive EDR's during successive groups of five trials during the "relaxed state" (R) and the "attentive state" (A).

Fig. 4. The mean frequency of positive pulse rate responses during successive groups of five trials during the "relaxed state" (R) and the "attentive state" (A).

Frequency of positive reactions. In Fig. 3 the mean phasic reactivity of the EDA is shown in terms of the number of positive reactions during successive groups of 5 trials. The curves resemble those obtained for the amplitude data in Fig. 1. However, the frequency measure seems to yield a clear-cut difference not only between the slopes of the two curves but also between their average levels.

The corresponding frequency measure of the pulse rate response illustrated in Fig. 4 also shows a considerably higher average level and a less pronounced decline during the "attentive" than during the "relaxed state".

To sum up this gross analysis, the results seem to confirm the predictions made on p. 260. The "attentive state" as compared with the "relaxed" tends to increase the average levels of the parameters and to counteract habituation of both the tonicity and the phasic reactivity.

Statistical analysis

EDA-data. To obtain a statistical evaluation of the possible changes occurring within sessions, Friedman two-way analyses of variance by ranks were used (cf. p. 261). The mean values of the variables during successive groups of five trials were ranked intra-individually. Thereupon the individual rank-numbers during each trial-group were summated. Table I shows the rank-sums derived from the values of the prestimulus EDA during the two experimental sessions. These sums give a similar view of the changes occurring within each session to

Table I. Friedman analyses applied to the sums of ranks obtained from the mean prestimulus EDA during successive groups of five trials during each session

Trial	1—5	6—10	11—15	16—20	χ_r^2	P
"Relaxed"	25.5	33.0	24.5	17.0	7.71	> 0.05
"Attentive".....	27.0	22.0	26.0	25.0	0.84	> 0.05

Table II. Friedman analyses applied to the sums of ranks obtained from the mean EDR amplitudes during successive groups of five trials during each session

Trial	1—5	6—10	11—15	16—20	χ^2_r	P
"Relaxed"	37.5	23.5	18.0	21.0	13.41	< 0.01
"Attentive".....	34.5	31.0	19.5	15.0	15.39	< 0.01

the corresponding curves in Fig. 1. The value of χ^2_r obtained under the "relaxed state" is on the border of significance ($\chi^2_r = 7.71$, the critical value being 7.82). During the "attentive state" the differences between the sums of ranks are quite small and consequently the Friedman analysis yielded a very low χ^2_r-value ($\chi^2_r = 0.84$) being far from significant.

To make possible a comparison of the changes of the prestimulus EDA occurring within each of the two sessions, the previously mentioned (p. 261) "change indices" have been computed. When a Wilcoxon matched-pairs signed-ranks test was applied to the difference between these indices it yielded T = 19 ($p > 0.05$). It may seem rather remarkable that the difference between the "change indices" was found to be statistically insignificant when the difference between the χ^2_r-values was so clear. A probable explanation is that the "change indices" during the "relaxed state" was heightened by the somewhat rising trend obtained during the first ten trials.

The difference between the average values of prestimulus EDA in all the trials during the two experimental conditions was statistically estimated by means of a Wilcoxon test. A clearly significant result was obtained ($T = 7$, $p < 0.025$).

Highly significant declining trends in the EDR amplitudes have been found under both experimental conditions (Table II). The Friedman analysis applied to the results obtained during the "relaxed state" yielded $\chi^2_r = 13.41$ ($p < 0.01$) and during the "attentive state" $\chi^2_r = 15.39$ ($p < 0.01$). The somewhat lower χ^2_r-value during the "relaxed state" seemingly contradicts the impressions derived from the corresponding curves in Fig. 1. The reason is probably that a great number of tied ranks was obtained under this condition. When the Wilcoxon test was applied to the differences between the corresponding "change indices" it yielded a T-value of 8.5 in the predicted direction ($p < 0.05$).

No statistically significant difference was found between the mean EDR amplitudes ($T = 19$; $p > 0.05$).

Pulse data. In Table III are shown the sums of ranks derived from the mean values of the prestimulus pulse rate during successive blocks of five trials. The rank-sums decrease during the "relaxed" and increase during the "attentive state". These trends are, however, not statistically significant ($\chi^2_r = 3.81$ in the first case and $\chi^2_r = 2.31$ in the other).

Table III. Friedman analyses applied to the sums of ranks obtained from the mean prestimulus pulse rate during successive groups of five trials during each session

Trial	1—5	6—10	11—15	16—20	χ_r^2	P
"Relaxed"	28.5	28.0	18.5	25.0	3.81	> 0.05
"Attentive".....	24.0	24.5	21.5	30.0	2.31	> 0.05

Table IV. Friedman analyses applied to the sums of ranks obtained from the mean pulse rate amplitudes during successive groups of five trials during each session

Trial	1—5	6—10	11—15	16—20	χ_r^2	P
"Relaxed"	30.0	30.0	20.0	20.0	6.0	> 0.05
"Attentive".....	30.0	20.0	26.5	23.5	3.27	> 0.05

A Wilcoxon test applied to the difference between the "change indices" of the same parameter yielded a T-value on the border of significance ($T = 12$, $p > 0.05$, critical value of T being 11).

The mean values of the prestimulus pulse rate showed a statistically significant difference between the two sessions ($T = 11$, $p = 0.05$).

The sums of ranks of the pulse rate response amplitudes shown in Table IV decrease during both sessions. These decreases were insignificant but a somewhat higher value of χ^2_r was obtained during the "relaxed state" ($\chi^2_r = 6.0$ and 3.27). The differences between the "change indices" and between the mean response amplitudes of the pulse rate were both clearly insignificant ($T = 26$ in both cases).

Frequency of positive reactions: Lastly an analysis of the phasic reactivity was also made in terms of the frequency of positive reactions during successive blocks of five stimuli. In Table V are shown the sums of ranks obtained for the frequency of EDR's during each session. In accordance with the corresponding amplitude data, χ^2_r turned out to be significant both during the "relaxed" ($\chi^2_r = 9.72$, $p < 0.05$) and the "attentive state" ($\chi^2_r = 8.91$, $p < 0.05$). Even the difference between the "change indices" of this parameter was clearly significant ($T = 8.5$, $p < 0.05$). The total number of positive EDR's was

Table V. Friedman analyses applied to the sums of ranks obtained from the number of positive EDRs during successive groups of five trials during each session

Trial	1—5	6—10	11—15	16—20	χ_r^2	P
"Relaxed"	36	22	21	21	9.72	< 0.05
"Attentive".....	29	32.5	21.5	17	8.91	< 0.05

Table VI. Friedman analyses applied to the sums of ranks obtained from the number of positive pulse rate responses during successive groups of five trials during each session

Trial	1—5	6—10	11—15	16—20	χ_r^2	P
"Relaxed"	28	30.5	22.5	19	4.89	> 0.05
"Attentive".....	25	25.5	27.5	22	0.90	> 0.05

used as a measure of the mean reactivity. Contrary to the mean response amplitudes, a significant difference between the sessions was obtained for this measure ($T = 5.5$, $p < 0.05$, two-tailed).[1]

As to the frequency of positive pulse rate responses the Friedman analyses (Table VI) yielded insignificant values both during the "relaxed" ($\chi^2_r = 4.89$) and during the "attentive state" ($\chi^2_r = 0.90$). Even the difference between "the change indices" was insignificant ($T = 19.5$). So far the results were in accordance with those obtained for the pulse rate response amplitudes. However, whereas the mean reactivity in terms of this amplitude measure yielded an insignificant difference between the sessions, the corresponding difference between the total number of positive pulse rate responses was found to be significant ($T = 8$, $p = 0.05$, two-tailed).

Discussion

The results obtained in the present investigation were in accordance with the predictions. The increased alertness brought about by the preoccupation with the sighting device of the pupillary camera significantly heightened the tonic activation level of both EDA and pulse rate and counteracted the habituation of these variables. As to the phasic reactivity the influence on the mean response amplitudes was insignificant. The habituation of the EDR amplitudes was clearly delayed during the "attentive state", but no significant effect was found upon the habituation of the pulse rate response amplitudes. The total number of positive reactions independent of amplitude was considerably higher during the "attentive state" than during the "relaxed state". However, it should be remembered that the total numbers of reactions were average values which were affected by the degree of habituation induced by the experimental conditions. It is seen from Fig. 3 and 4 that the curves derived from the frequency of positive reactions start from approximately the same level during both sessions. In fact neither the mean frequency of positive EDR's nor of positive pulse rate responses during the first 5 trials showed significant differences between the two experimental conditions. It is therefore clear that the greater total number of reactions obtained during the "attentive state" was a consequence of the delayed habituation induced by this condition.

[1] Since no directional hypothesis had been stated a two-tailed test of significance was applied.

The present findings are in strong support of the suggestion that the results obtained in the recent investigation (SCHOLANDER 1960) were influenced in a decisive way by the use of the sighting device itself. Evidently the alertness induced by such relatively simple methods may contribute towards diminishing the variability of autonomic measurements during repeated experimental sessions.

It should be emphasized, however, that preoccupation with the sighting device during longer periods requires continuous cooperation on the part of the subject. In other words, the subject must be motivated to do his best. It seems probable that one of the reasons why the previous investigation yielded such clear-cut results was that the subjects were told that failure to maintain the proper position in relation to the camera would spoil the recordings.

It is interesting to compare the present findings with those of LARSSON (1960). In this study it was found that the degree of "psychological significance" of an auditory stimulus correlated directly to the magnitudes of the startle blink response and the evoked potential in the EEG. The "psychological significance" was assumed to be low under auto-stimulation, moderate when the stimulus was presented under relaxation and high when it was used in a reaction speed experiment. However, the prestimulus heart rate and respiration rate were found to decrease in the order of auto-stimulation, reaction speed measurement and relaxation. The prestimulus EEG activity was desynchronized under auto-stimulation, with a large amount of alpha activity during the measurement of reaction speed and a smaller amount during relaxation. Thus when considering the different experimental conditions the same gradient of prestimulus activation was obtained in both EEG activity, heart rate and respiration rate.

The reaction speed measurement and the relaxed condition in the investigation of LARSSON have many similarities to the "attentive state" and the "relaxed state" in the present study. The results are also similar, viz. higher levels of prestimulus activation and a tendency towards greater phasic responses under the former than under the latter condition. However, it seems very unlikely that the "psychological significance" of the stimulus used in the present investigation was changed by the preoccupation with the sighting device. On the other hand, it is obvious that variation in the alertness is a proper correlate to the results obtained in both investigations. It might be objected that the small responses and the high levels of prestimulus activation obtained by LARSSON under auto-stimulation seems to contradict this conclusion. However, it should be remembered that the arousal value of a stimulus is much greater when it is administered in a randomized order by another person than when all surprise is eliminated by allowing the subject to release the stimulus himself. Accordingly the results obtained under auto-stimulation are not relevant in the present context.

It can be concluded that increased alertness may delay the habituation of

autonomic response elements and heighten the level of tonic activation. However, it should be emphasized that such conclusions are valid only within the limits imposed by the present approach. It is as yet too early to say whether the results are an expression of a general tendency.

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Intermittent and Continuous Running

(A further contribution to the physiology of intermittent work.)

By

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Abstract

CHRISTENSEN, E. H., R. HEDMAN and B. SALTIN. *Intermittent and continuous running. (A further contribution to the physiology of intermittent work.)* Acta physiol. scand. 1960. *50*. 269—286. — Intermittent running on a tread mill at a speed of 20 km/h (12.4 miles/h) is analysed and a comparison between this work and continuous running at the same speed has been done. The present results are in agreement with the assumption that stored oxygen plays an important role for the oxygen supply during short spells of heavy work. When running intermittent 6.67 km in 30 min (effective work 20 min and rest 10 min), a trained subject attained a total O_2 uptake of 150 l. With an O_2 uptake of 0.4 l/min at rest standing at the tread mill, or 4 l per 10 min of rest, 146 l O_2 are due to the 20 min of work. The actual uptake at work was only 101 l and if normal values are assumed during rest pauses, a deficit in oxygen transport of 45 l arises during the 20 min of actual work. This quantity will be taken up during the 120 rest pauses of 5 sec each. Two thirds of the oxygen demand during the 120 work periods of 10 sec each will accordingly be supplied by oxygen transported with the blood during work, and one third will be covered by a reduction in the available oxygen stores in the muscles, which in turn will be reloaded during the subsequent 5 sec rest periods. Respiratory and circulatory functions at intermittent and continuous running with special reference to maximal values are discussed. Research on intermittent work may open up a new field in work physiology.

In earlier investigations from this laboratory intermittent heavy work on the bicycle ergometer was analysed in respect to certain physiological functions (CHRISTENSEN 1956, 1960, ÅSTRAND *et al.* 1960 a and b). It was shown that the length of the individual work period is most critical, whereas the length of the rest pauses and the total work output might be of secondary importance as far as the physiological load is concerned.

Oxygen stored in the muscles, probably mainly in combination with myohemoglobin, was postulated to explain why a trained subject could perform intermittent, heavy work (2,520 kpm/min) with short spells of activity (10 sec) aerobically or practically so.

In the present investigation intermittent running on a treadmill at a speed of 20 km/h (12.4 miles/h) and with short spells of work is analysed in details and a comparison between this work and continuous running at the same speed has been done. Special attention has been payed to the circulatory and respiratory functions and to the blood lactic acid concentrations obtained at intermittent and continuous work.

Material and Methods

Two physically well trained, male subjects were used. One of them, R. H., was also a subject in the earlier experiments mentioned above. His age was now 29 years, weight about 72 kg and height 177 cm. His capacity for oxygen uptake at 5 to 6 min of work on the bicycle ergometer was 4.60 l/min or 64 ml/kg × min. B. S. was 24 years old, weight about 83 kg and height 187 cm. His maximal oxygen uptake at work on the bicycle ergometer was 5.60 l/min or 68 ml/kg × min.

The experiments were done at about 8 o'clock in the morning and the subjects were in fasting conditions. The treadmill, in horizontal position, was set at a speed of 20 km/h. The exact time for the work and rest periods was read from an electrical clock. Usually, however, the subjects run a definite number of steps for the different work periods of 5, 10 or 15 sec. For B. S. the number of double steps were 15, 30 and 45, whereas R. H. had a slightly higher frequency or 17—19, 34 and 51. The distance run in 5 sec corresponded to 27.8 m, in 10 sec to 55.6 m and in 15 sec to 83.3 m. Due to the short periods and to the high speed slight variations might occur, but they are of no importance for the general trend, even if they might have some slight influence on work efficiency.

Blood samples for lactate determinations were collected at 5 min intervals during pauses of 30 sec. This procedure too will have only a slight influence on the total O_2 uptake and other functions at intermittent work. Blood was taken from a prewarmed fingertip to secure full arterialisation. The analyses were done according to the method of BARKER and SUMMERSON (1941), modified by STRÖM (1949).

The expired air was collected in Douglas bags and gas analyses were done on a modified Haldane apparatus. Due to short spells of work and rest the expired air from a certain number of work or rest periods were collected in the same bags. For closer analysis the collection time was often cut down to 5 sec periods. The exact timing was done with two electrically activated stop watches, which were started or stopped from the threeway stop cock when the subjects' expiratory air at the end of an expiration was collected in the "work bag" or in the "rest bag".

Table I. B. S. intermittent running 20 km/h; work 15 sec, rest 10 sec

Time after start of experiment, min	Specimen of expired gas	$\dot{V}_E$, l BTPS	f	V_T, l BTPS	$\dot{V}_{O_2}$, l STPD	RQ
5— 8	W. 0—15 sec	108.5	32.9	3.30	5.06	0.80
5— 8	R. 0—10 sec	115.4	32.6	3.54	4.50	0.85
12—15	W. 0—15 sec	115.9	37.9	3.06	4.94	0.83
12—15	R. 0—10 sec	124.4	—	—	4.54	0.83
18—20	W. 0— 5 sec	123.0	40.6	3.03	4.49	0.85
18—20	W. 5—10 sec	125.9	48.2	2.61	5.07	0.79
18—20	W. 10—15 sec	140.4	50.7	2.77	5.31	0.82
27—29	W. 0—15 sec	138.8	48.2	2.82	5.06	0.82
27—29	R. 0— 5 sec	151.2	49.6	3.05	5.13	0.83
27—29	R. 5—10 sec	136.4	—	—	3.99	0.94

The heart rate was taken with an electrocardiographic pulse counter and every pulse beat was recorded with a four channel pen recorder (Kelvin & Hughes) with a paper speed of 10 mm/sec. Two channels were used for exact timing. Before running and immediately after rectal temperature was taken with a calibrated fever thermometer and the weight of the naked subject was determined with an accuracy of $\pm$ 50 g.

The room temperature was between 17° and 21° C with a humidity of around 50 per cent. To secure optimal sweat evaporation electrical fans were placed at a short distance from the subjects.

All experiments were done without any warming up exercise. When a work period started the experienced subjects jumped on to the running treadmill, and when the work period finished, they jumped off it to a standing position with one leg on each side of the running belt.

Results

I. *Intermittent running*

O_2 *uptake*

Table I gives an example of the sampling procedure, used for determining O_2 uptake and related functions. From the 5th to the 8th minutes after the experiment had started, the expired air from a number of work periods of 15 sec duration each was collected in the first Douglas bag. The corresponding O_2 uptake was 5.06 l/min. During the same interval the expired air from a number of rest periods of 10 sec each was collected in a second Douglas bag resulting in an O_2 uptake corresponding to 4.50 l/min. The following determinations from the 12th to the 15th minutes gave practically identical results, 4.94 l/min and 4.54 l/min respectively. Between the 18th and the 20th minutes a more detailed fractioning of the expired air was done. In one bag the expired air from the first 5 sec of work was collected, in a second bag air from the following 5 sec, and in a third bag air from the last 5 sec was collected. A marked increase in O_2 uptake is seen between the first (4.49 l/min), second (5.07 l/min)

Table II. B. S. intermittent running 20 km/h; work 5 sec, rest 5 sec

Time after start of experiment, min	Specimen of expired gas	$\dot{V}_E$, l BTPS	f	V_T, l BTPS	$\dot{V}_{O_2}$, l STPD	RQ
5— 6^{30}	W. 0—5 sec	101.6	28.0	3.63	4.41	0.82
5— 6^{30}	R. 0—5 sec	95.7	25.7	3.72	4.52	0.79
15—16^{30}	W. 0—5 sec	102.6	31.1	3.30	4.36	0.81
15—16^{30}	R. 0—5 sec	100.8	29.5	3.42	4.55	0.77
20—21^{30}	W. + R.	100.8	30.5	3.30	4.44	0.78
25—26^{40}	W. 0—5 sec	100.1	29.7	3.37	4.29	0.79
25—26^{40}	R. 0—5 sec	101.6	29.0	3.50	4.57	0.75

and third bag (5.31 l/min). For the whole period of 15 sec the result, 4.96 l/min, agrees closely with the two earlier determinations (5.06 l/min and 4.94 l/min) as well as with the final one (5.06 l/min) collected between the 27th and 29th minutes of the experiment. In a similar way the expired air from the rest periods between the 27th and 29th minutes was fractioned for the first 5 and the last 5 sec of the 10 sec rest periods. Here a marked decrease in O_2 uptake was found from the first 5 sec period (5.13 l/min) to the second one (3.99 l/min). But again the result (4.56 l/min) for the whole period of 10 sec agrees closely with the two earlier ones (4.50 l/min and 4.54 l/min).

Of interest is a comparison between the oxygen uptake (5.31 l/min) during the last 5 sec of the work period and the first 5 sec of the rest period (5.13 l/min). Apparently the O_2 uptake declines immediately when work stops.

The only experimental condition, where a higher oxygen uptake was found during the first 5 sec of rest compared to work, was when B. S. ran in 5 sec periods. Table II illustrates such an example. The maximal difference was 0.28 l/min or some 5 per cent. The reason for this discrepancy between the results of the 5 sec work periods for B. S. and the other results both with B. S. and R. H. can not be given.

Here again the stability of the results is remarkable. If the O_2 uptake for the work and rest periods are added for the three intervals referred to in Table II, the results are the following: 4.47 l/min, 4.46 l/min and 4.43 l/min, which again agree closely with the forth determination (4.44 l/min) after the 20th minute, where the expired air for work plus rest was collected in the same Douglas bag.

In Table III the results from an experiment with the highest work output are given. B. S. ran for 30 min with 10 sec of work alternating with 5 sec of rest. The total distance was 6.67 km. Of interest here is to notice that the lowest O_2 uptake was seen during the first 5 sec of work (4.44 l/min), second came the 5 sec of rest (average 4.92 l/min) and the highest value (5.60 l/min)

Table III. B. S. intermittent running 20 km/h; work 10 sec, rest 5 sec

Time after start of experiment, min	Specimen of expired gas	$\dot{V}_E$, l BTPS	f	V_T, l BTPS	$\dot{V}_{O_2}$, l STPD	RQ
5— 6^{15}	W. 0—10 sec	124.6	44.2	2.84	5.02	0.86
5— 6^{15}	R. 0— 5 sec	135.5	41.9	3.23	5.14	0.89
12—13^{15}	W. 0—10 sec	142.4	50.4	2.83	5.28	0.87
12—13^{15}	R. 0— 5 sec	137.4	52.4	2.63	4.90	0.87
20—21^{30}	W. 0— 5 sec	138.9	53.9	2.56	4.44	0.91
20—21^{30}	W. 5—10 sec	156.7	57.3	2.73	5.60	0.87
25—26^{15}	W. 0—10 sec	142.9	51.2	2.79	4.87	0.90
25—26^{15}	R. 0— 5 sec	143.7	43.3	3.32	4.71	0.86

corresponded to the last 5 sec of the 10 sec work period. This equals the highest oxygen uptake ever recorded with B. S.

Fig. 1 gives an illustration of the changes in oxygen uptake for the two subjects, when the periods of work and of rest are of identical length, 5, 10 and 15 sec. The total distance run in 30 min was always 5 km.

Table IV summarizes the different work and rest combinations used. The total O_2 uptake per minute (work plus rest) were determined in the way mentioned before, 7—12 Douglas bags were collected during work and rest periods from the fifth minute on. The results show, as expected, a marked increase in total oxygen uptake per minute with effective work time or total distance

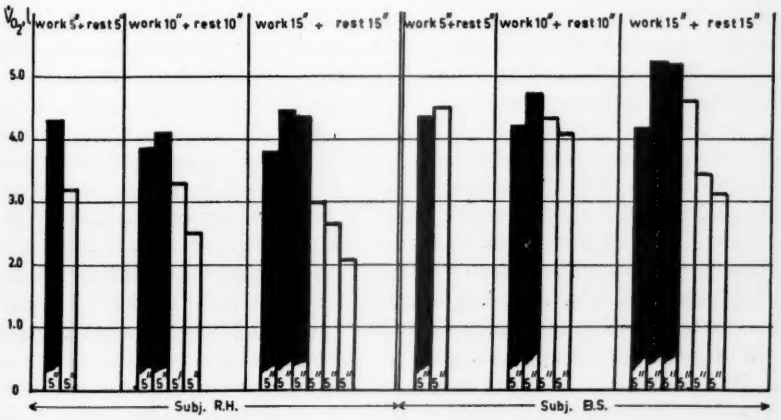

Fig. 1. Oxygen uptake at work (filled columns) and rest (unfilled columns) running 5 km at 20 km/h in 30 min as intermittent work with 5, 10 and 15 sec periods of work and rest. Subjects R. H. and B. S.

Table IV. O_2 uptake at different work and rest combinations for the two subjects, intermittent running, 20 km/h

Work period sec	Rest period sec	Work: rest	Distance run at each work period m	Number of runs in 30 min	Total distance run in 30 min km	Subject	$\dot{V}_{O_2}$, l net STPD	O_2 uptake l/km	Subject	$\dot{V}_{O_2}$, l STPD	Net O_2 uptake l/km
5	15	1:3	27.8	90	2.50	R. H.	2.16	22.2	B. S.	—	—
5	10	1:2	27.8	120	3.34		2.64	20.9		3.10	24.3
5	5	1:1	27.8	180	5.00		3.75	20.7		4.45	24.3
10	30	1:3	55.6	45	2.50		1.92	19.3		—	—
10	20	1:2	55.6	60	3.34		2.53	19.9		3.11	24.3
10	10	1:1	55.6	90	5.00		3.40	18.5		4.08	22.1
10	5	2:1	55.6	120	6.67		—	—		5.00	20.7
15	45	1:3	83.3	30	2.50		2.05	20.9		—	—
15	30	1:2	83.3	40	3.33		2.39	18.7		2.97	23.2
15	15	1:1	83.3	60	5.00		3.40	18.5		4.20	22.8
15	10	3:2	83.3	72	6.00		—	—		4.82	22.1

run in the 30 min experiments. There may be a slight tendency for a higher oxygen consumption with the short spells of work of 5 sec compared to 10 or 15 sec. But the differences are too small, and the possible errors in exact timing of the work periods are probably too large to allow any conclusive statements as to a statistical significant difference in running efficiency. The highest determined O_2 uptake for work plus rest was for R. H. 3.75 l/min, when the periods of work and rest were 5 sec each; when the periods were 10 or 15 sec, the O_2 uptake was 3.40 l/min. The corresponding values for B. S. were 4.45 l/min, 4.08 l/min and 4.20 l/min.

The higher O_2 uptake for B. S. compared to R. H., even when the distances run were the same for both, is mainly explained by the higher body weight of B. S., 83 kg compared to 72 kg for R. H. B. S. had an oxygen uptake of 400 ml/min when standing at rest with one leg on each side of the running belt, whereas R. H. had only 310 ml/min. The net O_2 uptakes in liters per km in Table 4 are calculated with a deduction of 310 ml/min for R. H. and 400 ml/min for B. S. Based on these values the average O_2 uptake per kg body weight and km was calculated to be 0.277 l for both. Obviously the efficiency in running at a speed of 20 km/h was the same for the two.

Both subjects reached O_2 uptakes during intermittent running close to or equal to their maximum. When running for 15 and resting for 15 sec R. H. reached 4.53 l/min, and when running for 10 and resting for 5 sec B. S. reached 5.60 l/min.

Pulmonary ventilation

Although oxygen uptake usually reached some sort of a steady state already from the 5th minute on, the other respiratory functions were at the highest work output less stable. Running for 15 sec and resting for 10 (Table I) the respiratory minute volume for the work periods increased for B. S. from 108.5 l to 138.8 l and for the rest periods from 115.4 to 143.8 l. The volume found for the last 5 sec of the work period was 140.4 l/min and for the first 5 sec of rest, 151.2 l/min. The respiratory frequency showed a steady increase from about 35 to about 50 per minute. A tendency for a decrease in tidal volume is also seen from Table I. The ventilation per liter of O_2 uptake showed during the latter part of the half hour an increase, at work from 21.4 l to 26.8 l, and at rest from 25.6 l to 34.2 l. One possible reason for this steady increase in ventilation was undoubtedly the blood lactic acid, which was 40 mg per 100 ml at the 5th minute and 66 mg per 100 ml at the 30th minute (comp. Table VI).

In Table III the highest pulmonary ventilation for B. S. at intermittent work, 156.7 l/min, is given; at continuous work as shown later the highest value was 158 l/min. For R. H. the corresponding values were definitely lower at intermittent work with a maximum of 107 l/min compared to 142.5 l/min at continuous work, where the blood lactic acid concentration was very much higher, which may explain some of this difference.

Heart rate and oxygen pulse

Fig. 2 gives an illustration of the recorded heart rates for R. H. when running at 20 km/h 2.5 km for 30 min with work periods of 5, 10 and 15 sec and with the corresponding rest pauses of 15, 30 and 45 sec. The heart rate for work belongs to the last 5 sec of this period; one rest value belongs to the first 5 sec, the other one to the last 5 sec of rest, which even means to the 5 sec preceding the following work period. It is clearly demonstrated that the heart rate at work and for the first 5 sec of the rest period are identical or practically identical.

This general finding for all work and rest combinations is of significance as to the reliability of judging the rate at work from pulse counts obtained during the first sec after work has stopped. In this laboratory a commonly used procedure is to take the exact time with a stop watch for 10 pulse beats immediately when work stops. Specially when using the heart rate as an indicator of physiological load in athletics or in industrial work, where pulse counting during actual work often is difficult or impossible, it is of importance to know, that post exercise values, when taken immediately after work, that is within the first 5 sec, are reliable indicators of the actual work situation, at least under normal climatic conditions.

Due to the fact that the oxygen uptake decreases immediately when work stops (exception see p. 272) and the heart rate stays unchanged for the first

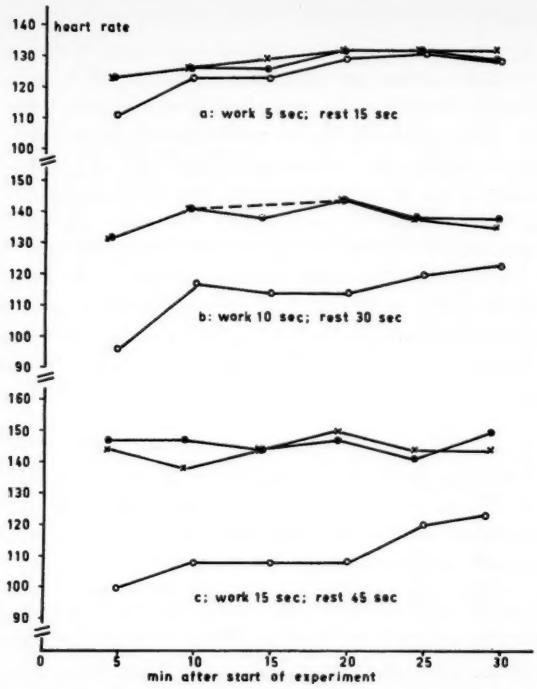

Fig. 2. Heart rate at different work and rest periods, intermittent work. Subject R. H. (×) last 5 sec of work period, (●) first 5 sec of rest period, (○) last 5 sec of rest period. The heart rate when standing at rest before the work experiments ranged 72—76 beats/min.

5 sec, an immediate decrease in O_2 pulse occurs. This decrease will go on until the next work period starts again.

Since a detailed fractioning in 5 sec periods during the work and rest periods has not always been done, a more complete calculation of the oxygen pulse at all different work and rest combinations could not be done. The available results show, however, that for subject R. H. the oxygen pulse was always lower during rest, even during the first 5 sec, than during the preceding work period. The same holds true for B. S. except for the 5 sec running periods, where a slightly higher oxygen pulse, 27.5 ml, was found during the first 5 sec of rest compared to 26.7 at work (running for 5 sec and resting for 10 sec). For both subjects similar maximal values for oxygen pulse were obtained at continuous and at intermittent work, for R. H. about 27 ml and for B. S. about 32 ml. (For further discussion on oxygen pulse at intermittent work see CHRISTENSEN 1960.)

Table V. Blood lactate concentrations at different time intervals intermittent running, 20 km/h. Subject R. H.

Work period sec	Rest period sec	Blood lactate, mg per 100 ml							Average
		Rest before work experiment	Min after start of work experiment						
			5	10	15	20	25	30	
5	20	18	14	12	9	11	11	8	11
5	15	13	18	21	14	14	11	11	15
5	10	—	18	17	16	13	10	9	14
5	5	10	42	50	51	48	45	45	47
10	30	9	19	15	14	16	13	11	15
10	20	14	20	16	19	19	16	20	18
10	10	16	46	53	47	43	39	42	45
15	45	13	15	12	13	14	13	14	14
15	30	18	32	30	24	30	32	23	29
15	15	14	62	72	74	79	82	80	75
	average	14	29	30	28	29	27	26	

Blood lactic acid

Table V and Table VI give the blood lactic acid concentrations at the different time intervals from the 5th to the 30th minutes of the experiments. The results from the single experiments are averaged and so are the determinations done at the fixed time intervals in all different experiments.

The results in Table V (subject R. H.) show that there is no, or only a slight increase in blood lactic acid concentrations at work compared to normal rest, when the periods of work are 5 or 10 sec, and the total distances run in 30 min are 3.33 km or less, or, when the work periods are 15 sec and the total distance is 2.5 km (work : rest = 1 : 3). With 15 sec work periods and a total distance of 3.33 km (work : rest = 1 : 2) a slight increase in blood lactic acid takes place during the first 5 min, and a level averaging 29 mg per 100 ml is seen for the following 25 min. If the total distance reaches 5 km (rest : work = 1 : 1) a more definite increase in blood lactic acid concentration is seen. When running for 5 or 10 sec this increase, however, only takes place the first 5 min of the experiments, from there on a level at an average of 47 and 45 mg per 100 ml respectively is found. When running for 15 and resting for 15 sec the increase is larger and continuous, 62 mg per 100 ml at the 5th minute and 80 mg at the 30th minute. This represents the only work situation examined with subject R. H. where anaerobic conditions are indicated for the whole experiment which, with a longer work time than 30 min, would limit his work performance.

Looking at the averaged values from all experiments for the 5th, the 10th

Table VI. Blood lactate concentrations at different time intervals intermittent running, 20 km/h. Subject B. S.

Work period sec	Rest period sec	Blood lactate, mg per 100 ml							Average
		Rest before work experiment	Min after start of work experiment						
			5	10	15	20	25	30	
5	20	—	13	8	9	7	7	9	9
5	15	14	20	13	9	10	10	12	12
5	10	16	14	14	15	14	21	17	16
5	5	12	28	23	20	22	23	24	23
10	30	12	11	11	11	10	11	10	11
10	20	17	22	18	27	27	25	23	24
10	10	15	19	18	17	21	17	25	20
10	5	14	42	43	38	49	47	47	44
15	45	9	13	9	16	11	12	10	12
15	30	23	21	16	16	14	16	14	16
15	15	15	—	18	19	19	23	28	21
15	10	11	40	42	48	54	54	66	51
average		14	22	19	20	21	22	24	

minute a. s. o., it is obvious that the blood lactic acid concentration is more likely to decrease than to increase from the 5th minute on. This is even more clearly shown when the determinations from the experiment with 15 sec work and 15 sec rest are excluded from the averages, then a decrease from 25 mg per 100 ml at the 5th minute to 20 mg at the 30th minute is seen.

The results in Table VI show the same general trend for subject B. S. with low lactic acid concentrations when the total distances run are 2.5 or 3.33 km. Even at a distance of 5 km the lactic acid level is low, averaging 23, 20 and 21 mg per 100 ml respectively when running for 5, 10 and 15 sec. The only work situation examined, where a definite increase in lactic acid between the 5th and the 30th minutes occurred, was when running a total distance of 6 km with work periods of 15 and rest periods of 10 sec. If the results from this last experiment are excluded from the average values in Table VI, the concentration at the 5th minute of work is 18 and at the 30th minute 17 mg per 100 ml.

Both subjects showed consequently a more marked tendency for increased lactic acid concentrations, anaerobic work, when the work periods were 15 sec compared to 5 or 10 sec.

Body temperature and heat regulation

When running a total of 5 km R. H. showed an increase in rectal temperature of 1.9° C in all three instances referred to in Table IV. The highest temperature, measured immediately after work, was 39° C. After running a

total of 6.67 km in 30 min B. S. showed an increase of 2.25° C and reached 39.2° C. The corresponding weight loss showed for R. H. an average of 0.58 kg and for B. S. with the higher work load 0.85 kg in 30 min.

A rough estimation will give some information about the heat balance in the case of B. S. At an oxygen uptake of 5.00 l/min, an average RQ of 0.88 (compare Table III) and a heat equivalent of 4.9 kcal per liter of oxygen, the total energy output in 30 min will be 740 kcal. If we assume, that the measured increase in rectal temperature of 2.25° C is representative for the whole body (83 kg) — the working muscles will have a somewhat larger and other tissues as the skin a lower increase, — 150 kcal will be stored in the body. If 0.8 kg of the total weight loss of 0.85 kg is due to evaporation, about 450 kcal are eliminated that way, some 10 per cent from the respiratory track and 90 per cent from the skin (compare the results of NIELSEN (1938)). Obviously the sweat rate has been of the order of 1.5 l/h, which undoubtedly is on the upper limit of what the sweat glands are supposed to handle at the actual climatic conditions. The high body temperature or the steep increase was subjectively not at all felt unpleasant. Unpleasant was, however, the profuse sweating from the face with sweat running into the eyes.

II. *Continuous running*

Continuous running at a speed of 20 km/h is even without wind resistance, as on the treadmill, a typical non steady state work, where the work time will be limited by an accumulation of anaerobic metabolites in the working muscles and in the organism as a whole. For the subject R. H. three minutes of continuous running was the limit for what he could perform. Five minutes after work had stopped, his blood lactic acid reached a maximal value of 151 mg per 100 ml (cf. Fig. 4). The other subject B. S. also went on for three minutes, but was not totally exhausted at the end. His blood lactic acid concentration also showed a maximum after 5 min of recovery and reached 117 mg per 100 ml indicating a close to but not maximal performance.

The values for O_2 uptake, pulmonary ventilation and heart rate are given in Fig. 3. *Oxygen uptake* showed a steep increase and a value corresponding to more than 4 l/min was reached 1 to 1.5 min after start of work for R. H., and more than 5 l/min for B. S. Already between 0.5 and 1 min the O_2 uptake for B. S. reached a value corresponding to 4.88 l/min. Both subjects reached an O_2 uptake very close to the earlier determined maximal values, for R. H. 4.54 l/min (max. 4.60 l/min) and for B. S. 5.55 l/min (max. 5.60 l/min) before the end of the three minutes' work period. For both subjects the O_2 uptake was increased some 18 times compared to the normal basal values and a tenfold increase took place during the first 0.5 min of work.

The *respiratory minute volume* (at B. T. P. S.) reached for B. S. 158 l between 2 and 2.5 min; the highest volume for R. H., 142.5 l, was measured between 1.5—2 min. At these high respiratory volumes B. S. had an electrically re-

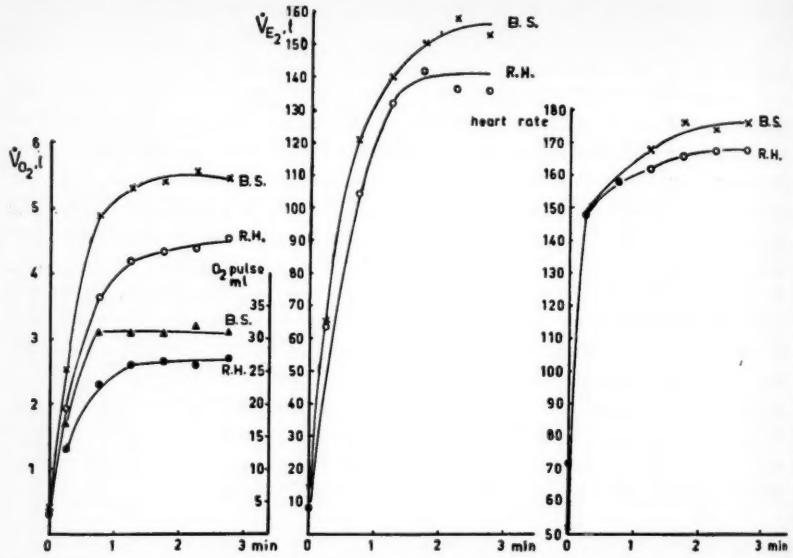

Fig. 3. Oxygen uptake, oxygen pulse, pulmonary ventilation and heart rate at continuous work (running 20 km/h for 3 min). Subjects R. H. and B. S.

corded *respiratory rate* of 48.9/min and a *tidal volume* of 3.23 l. R. H. had a rate of 48.2 and a tidal volume of 2.96 l. Per liter of oxygen the corresponding respiratory volumes varied between 24.9 l and 28.5 for B. S., and between 29.4 and 33.0 l for R. H. during the three minutes of work.

The *heart rate* showed for both subjects a steep increase during the first minute of work, a rate above 150 per min was reached during the first 0.5 min. For B. S. the rate levelled off around 175 per min, whereas for R. H. it levelled off below 170. This is an atypical pulse reaction for R. H., who usually would show maximal values of more than 180 at such a work load. The reason for this atypical reaction can not be given. An after control of the records showed no indication of experimental errors.

The calculated *oxygen pulse*, showed for B. S. a practically constant value of 31 ml, reached already 0.5 min after work had started; for R. H. the value of 26 ml was attained after 1 min of work.

For R. H. the average resting *blood lactate* in 9 determinations was 10.5 mg per 100 ml with the range of 6 to 15 mg per 100 ml. For B. S. the corresponding average was 12.5 mg per 100 ml in 8 determinations, range 8 to 18 mg per 100 ml.

After each work experiment six blood samples were taken, one during the first minute of recovery and five samples spread out over the following 10 to

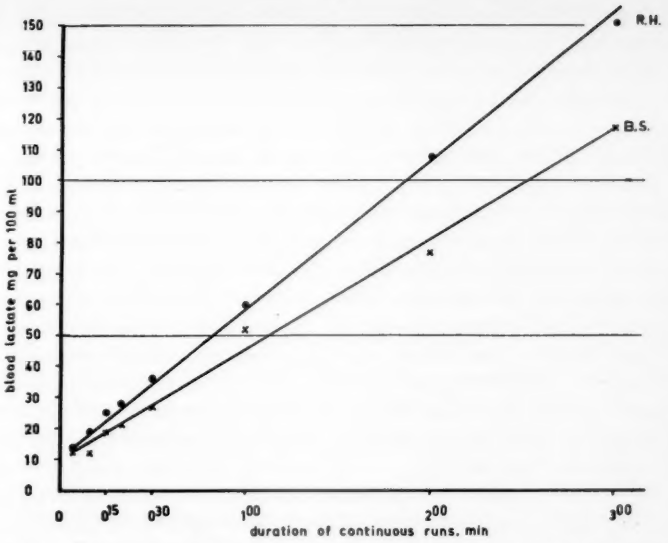

Fig. 4. Blood lactate concentrations. Maximal post-exercise values obtained during recovery after continuous running of different duration, from 5 sec to 3 min. Subjects R. H. (•) and B. S. (×).

15 min. The highest recorded post-exercise value for each experiment is given in Fig. 4.

The lactic acid concentration increases practically rectilinear, for R. H. roughly with 50 mg per 100 ml for each minute of work. Only after the 5 sec run no increase was found; the resting value before running was 15 mg per 100 ml and the post-exercise values were 10, 12, 11, 10, 10 and 14 mg per 100 ml. For B. S. the lactic acid increase per work min was roughly 40 mg per 100 ml. After running for 5 and for 10 sec all the post-exercise values were lower than the rest values before running. In the experiment with 10 sec running the rest value was 15 mg per 100 ml and the following post-exercise values were 10, 12, 9, 7, 8 and 9 mg per 100 ml. With a linear increase of 40 mg per cent per minute an increase of some 7 mg per 100 ml should be expected in this latter experiment, instead a slight decrease was found.

Discussion

The here mentioned results are in agreement with the assumption that oxygen stored probably mainly in combination with myohemoglobin in the working muscles, plays an important role for the oxygen supply during short spells of heavy work.

The "buffering" effect of the stored oxygen as to the total oxygen supply

during heavy intermittent work may be illustrated by the following example. When running 6.67 km in 30 min (work 10, rest 5 sec) B. S. had an average O_2 uptake (work plus rest) of 5.00 l/min or a total of 150 l. His effective work time was 20 min and he rested for 10 min. As his normal oxygen uptake at rest, standing at the treadmill, was 0.4 l/min, a total of 4 l has to be subtracted from 150 l to get the total O_2 demand of 146 l due to the 20 min of work, which corresponds to 7.3 l O_2 per work minute. The actual uptake per minute (cf. Table III) corresponded to 5.05 l/min during the work periods, or for the 20 min of work 101 l. Obviously a deficit in oxygen transport of 45 l arises during the 20 min of actual work, and this quantity is supplied during the 120 rest pauses of 5 sec each. The quantity that has to be repayed during each of the 120 rest pauses amounts to an average of 375 ml. With a demand of 7.3 l/min or 1.217 l per 10 sec two thirds will be supplied by oxygen transported with the blood during actual work, and if exclusively aerobic metabolism is assumed at work, one third will be covered by a reduction of the available oxygen stores in the muscles, which in turn will be reloaded during the subsequent 5 sec rest period.

In our earlier investigations (ÅSTRAND *et al.* 1960 b) with the subject R. H. we came to the conclusion that about 0.43 l O_2 ought to be available in the working muscles at the beginning of each work period. As subject B. S. definitely, in respect to physical work capacity, is the stronger of the two, the above mentioned calculations for B. S. indicate, that with 10 sec work periods he should have a fair margin for a further O_2 supply from the depots in the working muscles. With work periods of 15 sec this margin should, however, be reduced markedly, and the tendency shown for increased anaerobic condition with these longer work periods agrees with this assumption.

A further analysis of the results in Table VII, which were calculated in the same way as the before mentioned example, gives the following results.

Table VII. Calculated O_2 demand and O_2 deficit at different work and rest combinations. Intermittent

Subject	Work period sec	Rest period sec	Total work min	Total rest min	Average $\dot{V}_{O_2}$, l work + rest	O_2 demand for work l
R. H.	5	5	15	15	3.75	108.0
	10	10	15	15	3.40	97.35
	15	15	15	15	3.40	97.05
B. S.	5	5	15	15	4.45	127.8
	10	10	15	15	4.08	116.4
	15	15	15	15	4.20	120.0
	10	5	20	10	5.00	146.0
	15	10	18	12	4.82	139.8

For subject R. H. the same average blood lactic acid concentration is seen, 47 and 45 mg per 100 ml respectively, when the work periods were 5 and 10 sec; the O_2 deficit was, however, only 0.240 l per work period at 5 sec but 0.402 l at the 10 sec periods. At 15 sec periods the corresponding values were 75 mg per 100 ml and 0.570 l.

To find a possible explanation for the relatively high lactic acid values even at the 5 sec work periods a consideration of the average load and of the maximal load on the oxygen transport system may be of some significance. The average O_2 uptake per min for work plus rest (at 5 sec of work) was 3.75 l/min or 82 per cent of the maximal aerobic capacity for this subject. The actual uptake during the work period corresponded to 4.30 l/min or 94 per cent of the aerobic work capacity. At 10 sec work periods the average load on the oxygen transport system was 74 per cent and the highest load 89 per cent. This could give some explanation for the relatively high lactic acid values with the 5 sec work periods, and could of course also be valid for the results with the 15 sec work periods, where 99 per cent of the aerobic capacity is engaged during the latter part of the work periods. It is well known from earlier investigations, both from this and from other laboratories, that an increase in blood lactic acid concentration always takes place, especially during the first minutes of work, when the actual O_2 uptake in continuous work surpasses a certain percentage of the aerobic capacity.

The same explanation may fit the results with subject B. S. with exception of those from the experiment with 15 sec work and 15 sec rest periods (compare Table VI and VII). In this experiment the lactic acid stays constant or practically constant at an average concentration of 21 mg per 100 ml indicating aerobic or practically aerobic condition during the whole experiment. The relative load on the oxygen transport system corresponds to 75 per cent of the maximum, if the average O_2 uptake for work plus rest (4.27 l/min) is

running, 20 km/h.

O_2 demand per min of work, l	O_2 demand for each work period l	Actual $\dot{V}_{O_2}$, l at work	O_2 uptake each work period, l	O_2 deficit each work period, l	Max $\dot{V}_{O_2}$, l at work	Average blood lact. mg per 100 ml
7.20	0.598	4.29	0.357	0.240	4.30	47
6.49	1.082	4.08	0.680	0.402	4.10	45
6.47	1.618	4.19	1.048	0.570	4.53	75
8.52	0.710	4.35	0.363	0.347	4.40	23
7.76	1.293	4.38	0.730	0.563	4.71	20
8.00	2.000	4.54	1.135	0.865	5.34	21
7.30	1.217	5.05	0.842	0.375	5.60	44
7.77	1.943	5.02	1.255	0.688	5.31	51

considered, but as much as 95 per cent (or 5.30 l/min) if O_2 uptake for the last 5 sec of the work periods is considered.

It is quite remarkable that subject B. S. within 30 min can make 60 runs of 83.4 m each, with an O_2 demand corresponding to 8.00 l/min, and with a maximal O_2 uptake corresponding to 5.34 l/min, without hardly any increase in blood lactic acid. This can only be explained by an exceptional high ability to "flatten out" the work load over the subsequent rest period, which is indicated by the enormous deficit for transported oxygen of 0.865 l that he can compensate for at each work period of 15 sec duration.

In calculating the O_2 demand and O_2 deficit for the actual work periods as done in Table VII, we have assumed that the "rest O_2 uptake" is the same during the 30 min experiment as determined separately before the experiment, or 0.310 l/min for R. H. and 0.400 l/min for B. S. This assumption is not strictly justified. A number of physiological functions are highly elevated above the rest level, and this may involve a certain but indeterminable extra demand for oxygen, which is unrelated to a real deficit in uptake during actual work. The given values for O_2 deficit should therefore be taken as possible maximal values and they may not in a quantitative exact way be used for calculating the amount of stored oxygen, as we have done before. To our opinion the possible errors must, however, be relatively small. If the metabolism during rest pauses is much higher than assumed, this would involve a decrease in mechanical work efficiency at intermittent work. In two of our earlier publications (CHRISTENSEN, HEDMAN and HOLMDAHL 1960, and ÅSTRAND *et al.* 1960 a) where work efficiency at continuous and intermittent work on the bicycle ergometer were compared, such marked differences were not found. A tendency for a slightly lower efficiency at intermittent work was seen, however, even at short work and rest periods involving no or only a very slight increase in blood lactic acid. Part of this difference and perhaps the whole might be explained by the fact, that at intermittent work on the bicycle ergometer the subject has to accelerate the heavy flywheel from standstill at every work occasion. At continuous work the flywheel runs at a constant speed all through the experiment, which really means a somewhat lower work output and consequently a somewhat lower demand for O_2 uptake at continuous work compared to intermittent.

It is remarkable that at a work load asking for an O_2 uptake of 5.00 l/min (work plus rest) the RQ for the 30 min experiment averaged only 0.88 with a maximal deviation of 0.03 (cf. Table III). This and the other low values for RQ indicate, that in spite of the high work output the metabolism has been almost entirely aerobic, which is further confirmed by the relatively low and after the 5th minute usually stable blood lactic acid concentrations.

There are obvious differences between the two subjects with respect to their reaction to increasing length of the work periods. When running a total distance of 5 km the oxygen uptake for R. H. reached almost identical values,

at 5 sec 4.30 l/min, at 10 sec 4.09 l/min and at 15 sec 4.45 l/min. In all instances they are close to his maximum (4.60 l/min). When running the same distance B. S. had at 5 sec work periods 4.35 l/min, at 10 sec 4.71 l/min and at 15 sec 5.34 l/min (cf. Fig. 1). The relatively low value at 5 sec work is possible only because of the extremely high value, 4.50 l/min during the following rest period. For R. H. the corresponding value was only 3.20 l/min. When comparing the work and the rest periods for B. S. there is a definite trend towards more or less identical values for oxygen uptake during the short work and rest periods. Subject R. H. on the other hand shows a steep decrease in oxygen uptake as soon as work stops, even at the short work periods of 5 sec.

For several reasons the reaction of B. S. seems to be superior to that of R. H.; to a higher degree he will be able to "flatten out" the effect of the work load over the work and rest period. At the work period R. H. with an uptake of 4.30 l/min used 94 per cent, whereas B. S. with an uptake of 4.35 l/min only used 78 per cent of his maximum for oxygen uptake. The low lactic acid concentration, average 23 mg per 100 ml (cf. Table VI) in contrast to 47 mg (cf. Table V) for R. H., might be significant in this respect.

Especially the results from intermittent running with B. S. are of interest as to the problem of the respiratory control at work. Looking at Table I, II and III it may be difficult to decide whether a given pulmonary ventilation belongs to the work or to the rest periods. Changes in O_2 uptake and pulmonary ventilation are synchronized independently of work or rest, most likely with the concentration of metabolites and "oxygen demand". Therefore the pulmonary ventilation can not, at least not to any greater extent, be governed by nervous impulses, either radiating from the motorregion of the cortex cerebri or from proprioceptors in muscles, joints and tendons, which all should show a high activity during work but not at rest. Here again subject R. H. reacts somewhat differently. His pulmonary ventilation (and oxygen uptake) declines more abruptly when work finishes, even at the short periods of work.

The results of the experiments with continuous running at 20 km/h show without doubt that for subject R. H. the running time of 3 min, in which he covered a distance of 1 km, represented a maximal performance in respect to both his aerobic and anaerobic work capacity. For subject B. S. the running time and speed was sufficient to load his aerobic work capacity, or oxygen transporting system, to maximal values, but the blood lactate concentration was definitely below maximum, indicating a submaximal load on his anaerobic capacity. If we assume a maximal tolerable blood lactate value of 150 mg per 100 ml as for R. H., B. S. should be able to run for 4 min or cover a distance of 1.35 km, cf. Fig. 4.

It is difficult to settle if the low lactic acid concentrations found after a single short run has any significance as to aerobic or anaerobic conditions during the first 5 or 10 sec of work. The total production of anaerobic metabolites are of course relatively small due to the short work time, and a dilution by the

body fluids will anyhow result in low blood concentrations. Only direct determinations in the venous blood from the working muscles might give a definite answer to the question, whether a single run of 5 or 10 sec duration at a speed of 20 km/h can be performed aerobically due to oxygen stored in the muscles.

For further discussion as to the possible role of myohemoglobin as an oxygen store, the reader is referred to the earlier publications by ÅSTRAND *et al.* (1960 b). Independent of the validity of this assumption the following experimental findings are of significance.

Two physically trained subjects can run continuously for 3 respectively 4 min on the treadmill at a speed of 20 km/h, reaching maximal values for oxygen uptake and for blood lactic acid. At the end of this time when they have run a total distance of 1 and 1.3 km respectively they will be totally exhausted and will need a comparatively long time for recovery. Running at the same speed but intermittent with short spells of activity and rest, the character of work will change entirely; despite a marked decrease in oxygen uptake during the actual work periods, the work can be performed without or with only a comparatively slight increase in blood lactic acid concentration, indicating aerobic or practically aerobic work conditions. The trained subjects can stand an effective work time of 15 respectively 20 min within the experimental time of 30 min and run a total distance of 5 respectively 6.67 km without being totally exhausted.

We are inclined to think that the before and here mentioned results concerning intermittent work opens up a new field of research, and the results may have rather far reaching consequences for practical work studies. Too little emphasis may until now have been laid on the critical length of the active phases in intermittent work.

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Acta physiol. scand. 1960. 50. 287—293

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Passage of Solid Spherical Particles across the Blood-lymph Barrier

By

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Abstract

GROTTE, G., L. JUHLIN and N. SANDBERG. *Passage of solid spherical particles across the blood-lymph barrier.* Acta physiol. scand. 1960. *50.* 287—293. — Solid spherical particles (radius 300—700 Å) of methylmethacrylate marked with a fluorescent dye were administered to dogs by continuous intravenous injection in order to obtain a steady plasma concentration. Lymphatics were cannulated and lymph collected from four regions of the body: leg, liver, heart and bronchial lymphatics. The passage of particles across the blood-lymph barrier was measured by means of simultaneous concentration measurements in blood and lymph. Particles up to 700 Å radius readily passed into liver lymph with a lymph-plasma ratio of approximately 0.20 in the "steady state". No measurable amounts of these particles were found in the lymph from leg, heart or bronchial lymphatics. In these regions protein molecules of "effective diffusion radii" up to 120 Å pass into lymph. If the large proteins pass by "bulk flow" through water filled pathways the size of these pathways or "capillary leaks" would lie between 120—300 Å radius.

A large number of investigations have shown that the normal capillary wall permits the passage of blood cells, fine inanimate particles, proteins and other large molecules out to the extra-vascular fluid, from where they are to a large extent transported back to the blood via the lymphatics (cf. YOFFEY and COURTICE 1956, RUSZNYÁK, FÖLDI and SZABÓ 1957, COURTICE 1959 a, b). Most of these studies have however, been done with techniques where thermal

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or mechanical damage to these delicate structures may have occurred. Thus leg lymph collected with modern improved techniques using polythene tubing contains practically no cells at all (GROTTE 1956). From numerous studies on microcirculation problems KNISELY concluded that the normal capillary wall does not permit the passage of erythrocytes (KNISELY, personal communication, 1959).

In earlier studies it was shown (GROTTE 1956) that dextran of molecular weights up to 300,000 passes from plasma to lymph in various regions of the dog. Since lymph-plasma ratios for dextran molecules 60,000—300,000 molecular-weight were similar it was postulated that they pass by means of a "bulk" filtration process through preformed leaks in the capillary wall. The effective diffusion radii of dextran with a molecular weight of 300,000 has been calculated to approximately 120 Å. In the present investigation solid particles of 300—700 Å radius were used in order to find the upper dimension of such capillary leaks as estimated by the passage across the blood-lymph barrier.

Methods

Particles

Solid spherical particles prepared by emulsion polymerization of methyl-metachrylate and marked with a fluorescent dye were used. They were of the same batch as described in a previous report (Sh, JUHLIN 1956) in which the radii of the particles were about 500 Å (range 300—700 Å) and their HLB (hydrophilic-lipophilic balance) value about 40. The original particle suspension which contained 24 % by weight of solids was diluted 1/10 with 5.5 % sterile glucose solution. Of this diluted suspension 3—5 ml per kg of body weight per hour was injected by continous intravenous injection.

Experimental procedure

Mongrel dogs 1—3 years old, of both sexes and of average body weight 15—20 kg were used. For induction of anesthesia 30 mg/kg body weight Nembutal® was given intravenously and additional doses were given later when required. Cannulation of blood vessels and lymphatics was carried out as described earlier (GROTTE 1956). For the cannulation of heart and bronchial lymphatics the techniques described by DRINKER and co-workers were employed (DRINKER and YOFFEY 1941) with the exception that polythene tubing was used. The origin of the heart lymph was tested by the injection of blue dye, Patent Blue V, into the heart muscle (ALLISON and SABISTON 1958). The particles of methyl methacrylate were injected intravenously by means of a continuous injection apparatus described by ÖBRINK (1948). The lymph was then collected from the various regions for several hours.

Time table for a typical experiment

Time

00.00 Injection of Nembutal® i. v. 30 mg/kg of body weight. Intratracheal tube inserted.
00.15 Cannulation of artery and vein for injection and blood sampling.
00.20 Cannulation of lymphatics: leg, liver, heart, bronch.
00.45 Start continuous injection of particles. Blood and lymph samples taken.
Controls: respiration, pulse rate, arterial blood-pressure, lymph-flow, cell counts in lymph.

Fig. 1. Particles in plasma.
Magnification 24,000. Cr-shadowed.

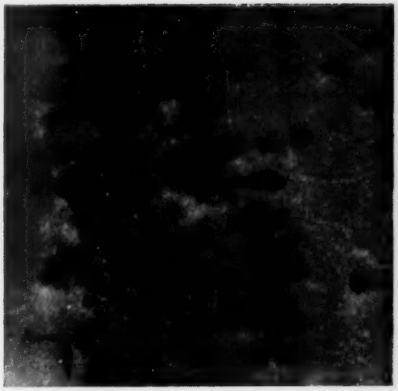

Fig. 2. Particles in liver lymph.
Magnification 24,000. Cr-shadowed.

Estimation of particles in blood and lymph

One millilitre of blood or lymph was added to 10 ml of acetone and shaken several times during 1 hour. This procedure dissolved the particles and the fluorescent colour. The fluorescence was then estimated in a Coleman Electronic Photofluorometer (JUHLIN 1958). Blank specimens of the blood and lymph were taken in each experiment before the injection of fluorescent particles. The subsequent estimations were corrected for these blank values which were practically negligible. The smallest detectable amounts in blood and lymph were 10 μg/ml.

Electronmicroscopic examination of particles in blood and lymph

To determine if the size-distribution of particles in lymph was the same as in plasma, electronmicrographs were made of some samples where the particles had been detected by the fluorescence determinations. After dilution 160 times with distilled water the sample was centrifuged for 2 hours at 1,200 r. p. m. The centrifuged plastic particles were placed on the preparation-net to dry. As may be seen in Fig. 1 and 2 the size distribution is about the same in lymph and plasma.

Results

Measurable amounts of particles already appeared in liver-lymph after about 10—20 min and the average lymph-blood ratio in 5 experiments during "steady state" conditions was estimated to be 0.20 (Fig. 3 and 4).

In lymph from the leg, heart or bronchial regions no measurable amounts of these particles could be detected (leg 5 exp., heart lymph 2 exp., bronchial lymph 1 exp.). With the method used the lymph-blood ratio should have been measurable if it exceeded 0.01.

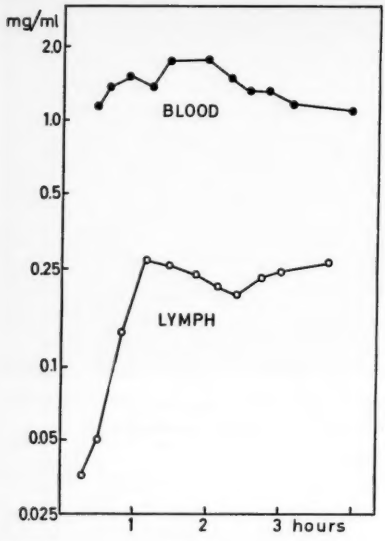

Fig. 3.

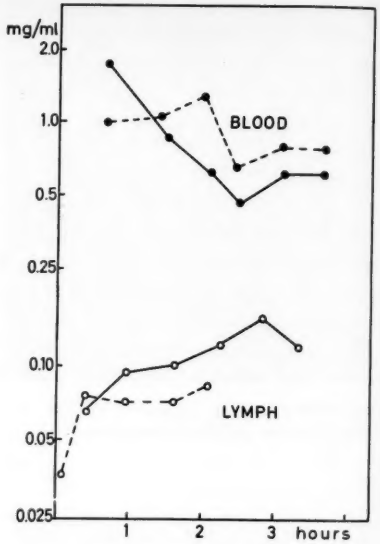

Fig. 4. (2 experiments)

Fig. 3 and 4. Concentration of particles in blood and liver-lymph.
Abscissa: Time in hours after starting particle injection.
Ordinate: Particles, mg/ml blood and lymph.

Discussion

In their extensive investigations on the permeability of blood capillaries (hind legs of cats and dogs) PAPPENHEIMER and co-workers (PAPPENHEIMER 1953) in order to explain the exchange of water soluble molecules across the capillary walls, concluded, that such capillaries have a mesh-work of "pores" of about 35—45 Å radius. Similar interpretations were drawn by GROTTE (1956), to explain the passage of dextran molecules (M 5,000—20,000) from blood to lymph in experimental animals. It has however long been known, that proteins in general leak through capillaries and are transported back to the circulating blood via the lymphatics. Similarly it has been found by many authors (WASSERMAN and MAYERSON 1952, BOLLMAN 1953, GROTTE 1956) that dextran molecules of up to 500,000 M pass into peripheral lymph.

It was found by DRINKER (cf. DRINKER and YOFFEY 1941) that the total concentration of protein in lymph varied in different regions. The protein concentration is highest in thoracic duct and liver-lymph. It is low in lymph from the legs, while lymph collected from the cervical duct or the intestines has a protein content intermediate between these two extremes. The protein

concentration ratio lymph/plasma (C_L/C_P) for each region is quite constant. Similar concentration ratios C_L/C_P for larger dextran molecules of M 60,000—300,000 were found by GROTTE (1956). When comparing protein distribution in lymph and plasma it has generally been found that in spite of the differences in total concentration, protein patterns in the two fluids are nearly identical, with a slight dominance of the albumin fraction in lymph. This may be due to a "diffusion-effect" *i. e.* at normal filtration rates, the linear velocity of water flow through capillary pores approaches or becomes slightly less than that of diffusing albumin molecules. (cf. PAPPENHEIMER 1953, GROTTE 1956). This means there may be an additional transport factor, *i. e.* diffusion of albumin across the capillary wall, resulting in a slight overrepresentation in the capillary filtrate for the albumin fraction. When comparing distribution curves of larger dextran molecules (M 60,000—350,000) in lymph and plasma, it was found by GROTTE (1956) that they also were practically identical and it was postulated that molecules of the size of albumin and larger are probably transported across capillary walls by a process of "bulk flow". The effective diffusion radii of these dextran molecules and the larger globulins are of the order of about 120 Å and the existence of some waterfilled pathways of at least these dimensions would seem plausible.

The present series of experiments were then undertaken to find the possible upper dimension of these pathways and particles of methyl-metachrylate seemed ideal since they only are about double the radius of the previously used test molecules and still only about 1/100 of that of an erythrocyte. It was found that our particles could pass from the liver sinusoids and to a large extent be transported out in the liver-lymph. This is in accordance with the finding that the particles are able to penetrate the sinusoidal membrane to the space of Disse and out into the bile (JUHLIN 1960). In the other regions investigated (heart, bronchial, cervical and leg-lymph) they were not found in the lymph in measurable amounts. Indeed it would seem possible that in these other regions they could have penetrated the blood capillary walls, but were trapped in the connective tissue and not transported away by the lymphatics. A most careful study of the tissues of animals infused with large doses of the plastic particles failed to reveal that they were trapped in the extravascular connective tissue regions.

In a recent publication MAYERSON *et al.* (1960) suggest from dextran experiments on dogs that capillaries may have two kinds of pores, but of larger dimensions than was found by GROTTE 1956. The differences in the results of these two investigations may to some degree lie in the different techniques used, *i. e.* measuring total concentration in lymph and plasma of certain "sharp" fractions of dextran versus the direct comparison of the distribution curves of the polymer in the two fluids. Every "sharp" fraction so far obtained will have a low and high molecular "tail", which will influence the results of permeability experiments.

These authors also discuss the possibility of "cytopemphis", *i. e.* transport by vesicles through living cells, as an explanation of capillary permeability. With the exception of the liver region our experiments do not support such a transport mechanism for the particles used in the study.

The interpretation of data concerning the passage of larger colloidal molecules through porous structures will also depend upon the degree of flexibility of the passing molecules. The information is here indirect and nonconlusive. Studies using artificial membranes have been unsuccessful due to "clogging" phenomena. Some information may be deduced from studies on dextran molecules solved in different water alcohol mixtures. In good solvents the dextran molecule is to a certain amount expanded but coils up to its unperturbed dimensions at the precipitation point. Measuring the radii of gyration (cf. FLORY 1953) at these two extremes, WALES and co-workers 1953 found differences of 25 % for molecular weight 500,000 but only 3 % for molecular weight 20,000. Molecules of radii 120 Å may be slightly flexible and pass through pores of somewhat smaller dimensions, but by using solid particles we think that these experiments have given information on the upper limit of capillary permeability.

The great permeability of the liver sinusoids has again been demonstrated in the present experiments. Data from earlier and present experiments on other capillary regions may be explained by water filled pathways or "leaks" in the capillary membranes of the order of magnitude of 100—300 Å radius. Our findings are to some extent in accordance with recent investigations using electron microscopic techniques (BENNETT *et al.* 1959). These large pores or "discontinuities" in the basement membrane demonstrated on the electron micrographs do not, however, explain the diffusion barrier which seems to exist for molecules of considerably smaller dimensions, indicating pore radii of 35—45 Å (cf. PAPPENHEIMER 1953, GROTTE 1956). It still seems questionable if the electron microscope *should* detect "pores" of such smaller dimensions (SJÖSTRAND, personal communication, 1959).

The particles were prepared and photographed at the laboratories of A.-B. Nobelkrut, Bofors, Sweden. Their valuable help is gratefully acknowledged.

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Oxygen Intake of Obese Individuals During Work on a Bicycle Ergometer

By

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Abstract

ÅSTRAND, I., P.-O. ÅSTRAND and A. STUNKARD. *Oxygen intake of obese individuals during work on a bicycle ergometer.* Acta physiol. scand. 1900. *50.* 294—299. — A group of 8 obese women and 4 obese men were examined for oxygen intake when working with submaximal loads on a bicycle ergometer. On 300 kpm/min the average for the obese women was 1.05 l/min, which was significantly higher than for a group of 28 non-obese subjects (0.89 l/min). The obese men had a higher oxygen intake than the control group (1.08 and 0.96 l/min respectively).

These findings suggest that increased mechanical efficiency does not play a part in the pathogenesis of human obesity.

This report deals with the oxygen intake during work of obese persons. It is generally taught that the mechanical efficiency of obese persons is the same as or less than that of persons of normal weight (RYNEARSON and GASTINEAU 1949 pp. 48—49, JOLLIFFE 1957 pp. 237—238). This teaching is apparently based upon only one report, that of WANG, STROUSE and MORTONK in 1930. Three years earlier GESSLER (1927) had reported that obese persons have a greater than normal mechanical efficiency. These are the only careful studies of this problem which we have been able to find.

Many studies indicate that the metabolic rate during work of normal adults can be fairly well predicted from the work load on a bicycle ergometer or from gross body weight during a step test or when walking (PASSMORE and

Table I. Characteristics of the subjects

subject	sex	age	body height cm	body weight kg	over weight[1] per cent	basal O_2 intake ml./min	
						determined	calculated
1	F	41	155	87	53	260	230
2	F	48	157	91	54	195	230
3	F	23	163	93	50	235	250
4	F	28	152	93	66	235	235
5	F	25	159	97	62	—	245
6	F	25	167	104	60	—	260
7	F	27	165	125	95	280	280
8	F	48	169	130	95	310	280
9	M	55	176	100	32	—	270
10	M	36	175	113	51	—	305
11	M	61	177	113	47	—	280
12	M	59	171	134	86	—	295

[1] = per cent overweight, as calculated from data given by the Metropolitan Life Insurance Company.

DURNING 1955, P.-O. ÅSTRAND 1956). This indicates a small individual variation in mechanical efficiency with sex and age. Very obese subjects were not included in those studies. In the course of the studies of human physical work capacity under a variety of conditions an attempt has been made to investigate the energy output of obese individuals when performing standardized muscular work.

Material and Methods

Twelve obese and 53 non-obese subjects were studied. Eight obese women and 4 obese men, and 25 male control subjects were studied at the Lankenau Hospital, while 28 non-obese female control subjects were studied at the Kungl. Gymnastiska Centralinstitutet in Stockholm. Average ages of the obese group were 33 for the women and 53 for the men, while ages of the control group averaged 34 for the women and 44 for the men. Mean per cent overweight of the obese subjects averaged 62 with a range from 32 to 95. Values for individual subjects are given in Table I.

All obese subjects were studied in the General Medical Clinic of the Hospital of the University of Pennsylvania to exclude any condition which might interfere with performance of the test. Because of the evidence that mechanical efficiency is lowered by a shift from carbohydrate to fat metabolism (P.-O. ÅSTRAND 1956) no subjects were on restricted diets at the time of the test.

Subjects 3 and 5 are of particular interest. These women had been previously studied for many months on the metabolic service of another hospital. During investigation under rigidly controlled circumstances each had progressively failed to respond to weight reduction diets and ultimately came to apparent caloric equilibrium on a diet

Table II. Results from work on a bicycle ergometer with a load of 300 and 450 kpm/min

No.	300 kpm/min					450 kpm/min				
	O_2 intake l/min STPD	mech. eff. per cent	heart rate	lactic acid mg per 100 ml	pulm. vent l/min BTPS	O_2 intake l/min STPD	mech. eff. per cent	heart rate	lactic acid mg per 100 ml	pulm. vent. l/min BTPS
1	1.16	15.4	131	28	32					
2	0.93	20.5	104	18	32					
3	0.98	19.6	132	29	26	1.24	21.6	155	35	32
4	1.10	16.5	127	19	28	1.44	17.8	145	25	33
5	0.93	21.1	145	28	26					
6	1.06	17.9	120	19	30	1.35	19.7	134	36	37
7	1.07	18.2	115	15	28	1.46	18.2	136	25	37
8	1.17	16.1	127	35	40					
9	0.90	22.8	95	14	22					
10	1.05	19.1	115	17	27					
11	1.03	19.1	92	23	30					
12	1.32	14.1	98	15	37					

containing 800 kcal. Although their basal metabolic rate decreased during this time, the decrease was not considered sufficient to account for their apparently greatly lowered caloric expenditure. One explanation of their low caloric requirement was that they might be functioning at an increased mechanical efficiency.

Work was performed on a bicycle ergometer with 50 pedal revolutions per minute. The mechanical efficiency in per cent was calculated from the formula:

$$\text{net mechanical efficiency (in per cent)} = \frac{\text{work performed} \times 100}{\text{total} - \text{basal energy expenditure}}$$

The numerator of the fraction is the caloric equivalent of mechanical work in kpm performed on a bicycle ergometer. (One kpm is the equivalent of 7.23 foot pounds and 0.00234 kcal.)

Total energy expenditure was calculated from the oxygen intake during work. After five minutes of a scheduled 10 minute work period expired air was collected in two successive DOUGLAS bags via a mouth piece and a low resistance respiratory valve. The volume of expired air was measured in a balanced spirometer and samples were analyzed by the HALDANE technique (PETERS and VAN SLYKE 1932 pp. 84—118). Similar values for oxygen intake were obtained on the first and second bags indicating that measurements were made in a steady state. The mean of the two values is accordingly reported. The test was repeated at an interval of two weeks on 10 of the obese subjects. The mean oxygen intake for the group on the two tests was 1.04 and 1.05 l/min, and no significant difference was found between the values of the two tests in any individual. The mean of the two tests is reported.

Basal energy expenditure was estimated from body surface and the Mayo Foundation Standard (BOOTHBY, BERKSON and DUNN 1936). Basal energy expenditure was measured directly in six cases and, as can be seen in Table I, compared favorably with the estimated value. The caloric coefficient of oxygen was set at 4,900 kcal/l during work and 4,825 kcal/l at basal condition.

As an additional assurance that measurements were performed in a steady state,

Table III. Summary of results on obese and non obese subjects. The statistical data give mean value, standard error of the mean and standard deviation. n = number of subjects

	n	300 kpm/min				n	450 kpm/min			
		O_2 l/min	mechanical efficiency per cent	heart rate	lactic acid mg per 100 ml		O_2 l/min	mechanical efficiency per cent	heart rate	lactic acid mg per 100 ml
obese women	8	1.05±0.04 0.10	18.2±0.8 2.1	125±5 13	24±3 7	4	1.37±0.05 0.10	19.3±1.0 1.9	143±5 10	30±3 6
obese men	4	1.08±0.09 0.18	18.8±1.8 3.6							
control women	28	0.89±0.01 0.06	21.0±0.3 1.6	118±3 14	17±1 4	28	1.17±0.01 0.06	22.5±0.3 1.2	137±4 18	26±2 9
control men	25	0.96±0.01 0.06	20.1±0.3 1.6	100±2 11	17±1 5	17	1.25±0.01 0.06	21.5±0.3 1.3	111±2 10	25±2 6

lactic acid was measured on each subject at the end of the work. Two or three samples of finger tip blood from a hand prewarmed in water were taken at two minute intervals and were analyzed by a modification of the method of BARKER and SUMMERSON (STRÖM 1949).

All subjects were tested on 300 kpm/min. Of the obese ones only some were also tested on 450 kpm/min. The studies of the non-obese subjects included determination of maximal oxygen intake and were reported by one of the authors (I. ÅSTRAND 1960).

Results

Table II gives the individual data obtained on the obese subjects and Table III summarizes the main results from obese and non-obese subjects.

Of the eight obese women tested on 300 kpm/min the gross oxygen intake averaged 1.05 l/min giving a mechanical efficiency of 18.2 per cent. Blood lactic acid was 24 mg per cent and heart rate was 125 beats per minute. The average mechanical efficiency of 18.2 per cent is significantly lower than the value of 21.0 obtained on the female subjects without evident obesity ($P < 0.01$). The difference in gross oxygen intake is highly significant ($P < 0.001$).

The same trend was observed among the four men tested at the same work load and the four women tested at a higher work load. The oxygen intake of the four men was 1.08 l/min and the mechanical efficiency was 18.8 per cent as compared with values of 0.96 l/min and 20.1 per cent respectively for 25 control subjects in tests performed at 300 kpm/min. At a load of 450 kpm/min the mechanical efficiency of four obese women was 19.3 per cent as compared

with a value of 22.5 for the control subjects. The small size of the samples precludes statistical analysis.

The blood lactic acid levels of each subject are listed in Table II. All values are considered sufficiently low to exclude the possibility of an oxygen debt during the collection of expired air (P.-O. ÅSTRAND 1952 pp. 92—102).

Discussion

This study indicates that oxygen intake during work is not lower and the mechanical efficiency of obese persons is not greater than normal. There is even evidence that the mechanical efficiency may be somewhat reduced. These results confirm the work of WANG *et al.* (1930) and are in opposition to those of GESSLER (1927).

The finding of a lowered mechanical efficiency in obese persons although statistically significant, seems of far less practical significance than the indication that obese persons are not more mechanically efficient than persons of normal weight. For these results fail to provide empirical evidence for the intriguing theoretical possibility — that obese persons can achieve a caloric surplus on a caloric intake and output which produce a caloric equilibrium in non-obese persons. It is, of course, possible that a few unusual obese persons may have an increased mechanical efficiency which plays a part in their obesity, and the occasional reports of obese persons with very low caloric requirements have aroused such suspicions. The negative findings in subjects 3 and 5 are therefore particularly interesting, since they represent two of the most carefully studied examples of persons who can apparently maintain their obesity on a very low caloric intake.

Two possible weaknesses of this study merit consideration. First, the female control subjects were from a different population and were studied in a different location. However, the study of the physical work capacity of large numbers of persons on both sides of the Atlantic has given no indication of differing mechanical efficiencies, and the experiments were carried out by the same investigators utilizing the same equipment.

Secondly, since the basal metabolic rate has been estimated from data on body surface, and not measured, the authors are aware that the values given on mechanical efficiency are uncertain. Since excessive body fat is metabolically inert the figure for basal metabolism, when calculated in such a way, might be too high and the estimated mechanical efficiency also too high. If so, the difference between the obese and the non-obese groups is greater than the one presented in Table III.

It should be emphasized that none of the obese subjects was on a restricted diet on the days preceding the work test. It is therefore assumed that the metabolism with regard to proportions between fat and carbohydrate combustion was normal. No efforts were made to determine the exact respiratory

quotient but the values were within the same range for obese as for non-obese subjects averaging 0.94 for both groups on 300 kpm/min.

A probable explanation for the higher energy output of obese individuals is the great amount of adipose tissue that is moved when exercising on the bicycle ergometer. It was especially apparent in subject no. 12 (body weight 134 kg) how the pendulous abdomen had to be moved from side to side during exercise.

Due to the great individual variation in oxygen intake of obese individuals when doing muscular work it is not advisable to predict the energy metabolism from the work load if respiratory and circulatory functions are studied. The actual energy production should be measured.

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Acta physiol. scand. 1960. 50. 300—305

From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden

The Influence of Anoxia on 48/80-Induced Histamine Release from Cat Skin

By

BARBRO WESTERHOLM

Received 6 July 1960

Abstract

WESTERHOLM, B. *The influence of anoxia on 48/80-induced histamine release from cat skin.* Acta physiol. scand. 1960. *50*. 300—305. — The investigation reported here was designed to determine whether oxidative energy is necessary for histamine liberation in the cat as in the guinea pig and the rat. The experiments were performed *in vitro* on finely chopped cat skin which was incubated with the histamine liberator, compound 48/80, during oxygenation and during anoxia. From the results it is evident that anoxia inhibits histamine release from cat skin, while glucose abolishes the inhibition. The findings suggest that histamine release from such tissue is a process requiring energy from oxidative or glycolytic processes.

The mechanism of histamine release by compound 48/80 in the cat, as in the rat, shows many similarities to anaphylactic histamine release in the guinea pig, as pointed out by CHAKRAVARTY (1959). A difference was noted, however, in regard to oxygen requirements: Histamine liberation from sensitized guinea-pig lung required the presence of oxygen, whereas anoxia had no inhibitory effect on histamine release from cat or rat tissue (CHAKRAVARTY, HÖGBERG and UVNÄS 1959, CHAKRAVARTY 1960). DIAMANT (1960) subsequently demonstrated, however, that anoxia inhibited the histamine liberating power of antigen, compound 48/80 and extracts from *Ascaris suis* in rats, if glucose was not present in the incubation fluid. When glucose was added to the solution histamine release occurred normally despite oxygen lack.

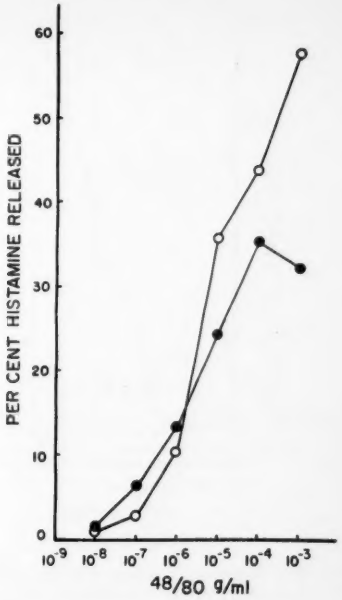

Fig. 1. Influence of oxygenation on histamine release from cat skin caused by compound 48/80.
○—○ = oxygenated samples;
●—● = non-oxygenated samples.

Experiments on rats and guinea pigs accordingly suggest that histamine release in those species is an active metabolic process. The same may as well be true of cat tissue, since histamine release therefrom can be blocked by the phosphorylation inhibitor, dinitrophenol (WESTERHOLM 1960). To ascertain the validity of this assumption regarding cat tissue, the following investigation was conducted.

The studies were performed *in vitro* on finely chopped cat skin. The skin was incubated with the histamine liberator, compound 48/80, during oxygenation and during anoxia and the effect of glucose on the release was studied.

Methods

The method employed was the same as that described in an earlier paper (WESTERHOLM 1960). Chopped cat skin in samples of 1 g was incubated in 25 ml beakers each containing 5 ml incubation solution consisting of 1.54×10^{-1} M NaCl, 2.7×10^{-3} M KCl and 9×10^{-4} M $CaCl_2$ (anhydrous). The pH was adjusted to 7.25 by addition of 10 per cent Sörensen phosphate buffer. Incubation was done at 37° C and oxygen was introduced through cannulae in the stoppers of the incubation beakers. In the anoxia experiments nitrogen was substituted for the oxygen.

Compound 48/80, a polymer amine, was used as liberator. The tissue was preincubated for 15 min in oxygenated or oxygen-free (nitrogen-saturated) incubation solution before adding the liberator, the incubation then being continued for a further 30 min. All tests were run in duplicate.

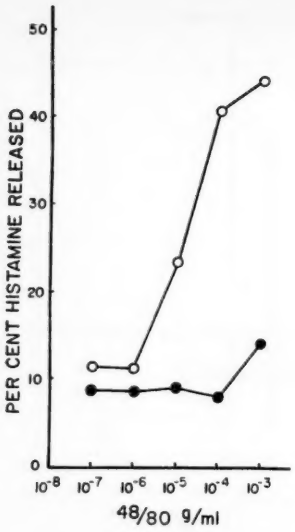

Fig. 2. a.

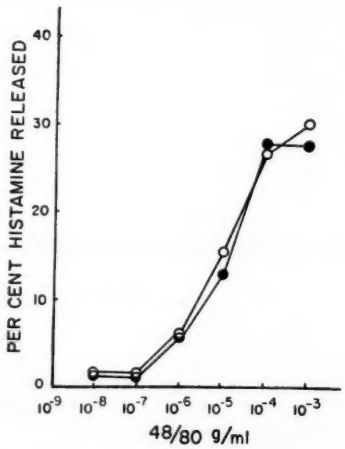

Fig. 2. b.

Fig. 2 a. Influence of oxygen and nitrogen respectively on histamine release from cat skin caused by compound 48/80. Incubation carried out in glucose-free solution. ○—○ = oxygenated samples; ●—● = nitrogen-treated samples.

Fig. 2 b. Influence of oxygen and nitrogen respectively on histamine release from cat skin caused by compound 48/80. Incubation carried out in the presence of glucose (5.6×10^{-3} M). ○—○ = oxygenated samples; ●—● = nitrogen-treated samples.

The liberated histamine was assayed on atropinized (1.5×10^{-6} M atropine sulphate) guinea-pig ileum. By adding mepyramine (5×10^{-7} M) to the intestine bath, it was shown that the intestinal contractions caused by the incubation solution were due to histamine.

Residual histamine in the tissue was determined by heating the pieces in N HCl (5 ml/g) at boiling point for 5 min. The extract was neutralized and brought up to suitable volume for testing on guinea-pig ileum.

The histamine released in the various experiments is expressed as per cent of total amount of histamine in the tissue. All values given in the figures are expressed as means of duplicate determinations.

Results

In the original method no extra oxygen was administered during incubation, the only oxygen present being that above the incubation fluid in the beakers. Since the oxygenation in these experiments might have been insufficient for the histamine release to proceed at optimal conditions, dose-effect curves obtained with and without additional oxygen administration were compared. Fig. 1 shows that the curves were parallel up to a concentration of 10^{-4} g 48/80 per

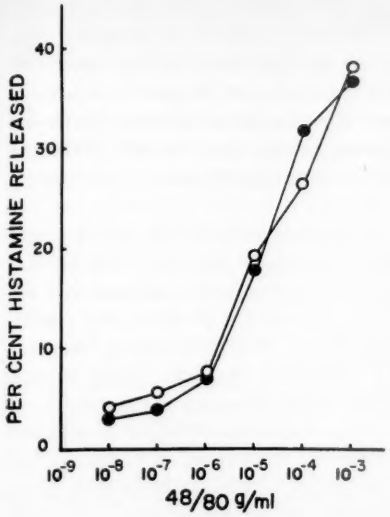

Fig. 3.

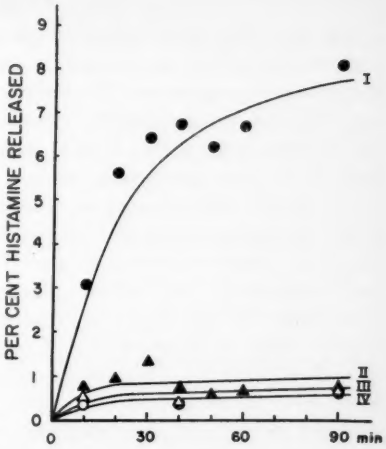

Fig. 4.

Fig. 3. Influence of glucose (5.6×10^{-3} M) on histamine release from cat skin caused by compound 48/80 during concurrent oxygenation. ○—○ = samples incubated in the presence of glucose; ●—● = samples incubated in glucose-free solution.

Fig. 4. Time curve for histamine release from cat skin caused by compound 48/80.
1. During oxygenation: I: ●—● = 48/80, 10^{-5} g/ml; II: ○—○ = controls.
2. During administration of nitrogen: III: ▲—▲ = 48/80, 10^{-5} g/ml; IV: △—△ = controls.

ml. At that point the histamine liberation in the unoxygenated samples reached its maximum, but in the oxygenated samples additional histamine was released.

Histamine release during oxygenation and during complete anoxia was thereafter compared. From Fig. 2 a it is evident that in anoxia no histamine release above the spontaneous liberation was obtained until the dose rose as high as 10^{-3} g 48/80 per ml.

If, on the other hand, the incubation was done in a solution containing 5.6×10^{-3}M glucose the histamine release was as high during anoxia as during oxygenation (Fig. 2 b).

The influence of glucose on histamine liberation during oxygenation was also investigated, and Fig. 3 shows the dose-effect curves for incubation with and without glucose. There was no significant difference between the curves.

In order to ascertain if the duration of incubation affected the histamine release during anoxia, a time curve with 10^{-5} g 48/80 per ml was plotted. Fig. 4 shows that after 90 min there had still been no liberation in the samples incubated during administration of nitrogen.

Discussion

It is clear from the results that release of histamine from cat skin produced by compound 48/80 is inhibited by anoxia provided that no glucose is present in the incubation solution. This finding, as well as the fact that the phosphorylation inhibitor, dinitrophenol, blocks histamine release from cat skin (WESTERHOLM 1960), suggests that histamine liberation from that tissue requires energy from oxidative or glycolytic processes.

The results differ from those obtained by CHAKRAVARTY *et al.* (1959), who failed to produce inhibition of histamine release from cat skin with anoxia. However, CHAKRAVARTY *et al.* used Tyrode's solution for incubation, and the fact that such solution contains glucose may account for the divergent results. The present findings agree, on the other hand, with those recorded by CHAKRAVARTY (1960) in guinea pigs and those obtained by DIAMANT (1960) in rats, as well as with the observations on isolated mast cells and mast cells in rat mesentery (HÖGBERG and UVNÄS 1960, UVNÄS and THON 1960). It is probable that the energy-requiring processes essential for liberation of histamine are localized to the mast cells, since degranulation of, and histamine release from, isolated mast cells can be blocked by metabolic inhibitors such as cyanide, thyroxine, and dinitrophenol. Anoxia of long duration also has a blocking effect (UVNÄS and THON 1960).

The investigation has revealed, moreover, a difference between the dose-effect curves with and without oxygenation, the discrepancy arising only after the higher doses of compound 48/80. Without additional oxygen administration the histamine liberation reached its maximum at 10^{-4} g 48/80 per ml. With oxygenation, however, maximal response had still not been reached at 10^{-3} g 48/80 per ml. — Higher doses were impracticable because such concentrations had a toxic action on the guinea-pig ileum. — The explanation may be that in the former instance the histamine release ceases when the oxidative energy processes are exhausted, while in the latter case the liberation may continue since oxygen is continuously administered.

In this connection it should be observed that the dose-effect curve in some experiments with oxygenation reached its maximum at a concentration of 10^{-4} g 48/80 per ml, but in others it did not do so until higher doses were administered. The histamine content of the skin was substantially greater in the latter than in the former instances and it seems possible that the amount of releasable histamine varied in the same way. Thus to obtain maximal histamine release from the tissue, lower doses of 48/80 would be required in the animals with smaller than in those with larger amounts of releasable histamine. — That cat skin seems to contain both releasable and unreleasable histamine has already been pointed out by PERRY (1956) and WESTERHOLM (1960).

It should also be noted that in some experiments it was possible to release about 30 per cent of the histamine in the tissue, in others 50 per cent or more.

This observation suggests that the proportion releasable histamine to unreleasable histamine in cat skin varies from one animal to another.

Anoxia appears to inhibit completely the liberation of histamine, as shown by both the dose-effect experiments and by the time-curve studies. After 90 min there had still been no histamine release with 10^{-5} g 48/80 per ml. At a dose as high as 10^{-3} g 48/80 per ml there was, however, a slightly greater release than in the controls after 30 min. The mechanism of this releasing action is obscure.

The experiments thus lend support to the assumption that histamine release from cat skin produced by compound 48/80 is a process requiring energy, and that the latter may be obtained from oxidative or glycolytic processes, just as has been found in the rat.

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Acta physiol. scand. 1960. 50. 306—310

From The Clinical Laboratory, The Royal School of Dentistry, Stockholm, Sweden

Excretion of Bromide Ions by Human Urine

By

RUNE SÖREMARK

Received 7 July 1960

Abstract

SÖREMARK, R. *Excretion of bromide ions by human urine.* Acta physiol. scand. 1960. *50.* 306—310. — The excretion of radio-bromide by the urine after a single dose of Br^{82} was studied in thirteen healthy students of about 22 years of age. The radioactive isotope, Br^{82}, was given orally in form of ammonium bromide in water solution. Urine specimens were collected about every second hour for five days. The concentration of Br^{82} in the urine specimens was measured and the excretion of Br^{82} per day has been tabulated. Also the cumulative excretion of radio-bromide was calculated and has been graphically demonstrated. The concentration of Br^{82} in blood and saliva was also measured. Venous blood samples were drawn from six of the subjects twice a day. Saliva samples were collected from these six students at about every fourth hour. The mixed saliva was paraffin stimulated. The biological half-life of bromide was equal in blood, urine, and saliva, about 12 days. Nearly all of the radio-bromide administered was found to be excreted by the urine. The mean concentration of Br^{82} in urine and saliva was about equal and 150 % of the blood level. It was found to exist a diurnal variation in the excretion of bromide by the urine. During sleep it was thus found that hardly any bromide was excreted.

Literature concerning the excretion of bromide ions from the human body is very scanty and the results from different laboratories do not always agree. The general purpose of the clinical experiments described in this paper was, therefore, to collect data on the excretion of bromide ions by the urine in healthy human beings. In a previous study (SÖREMARK 1960) on the distribution and kinetics of bromide ions in mice, rats, and rabbits the main way of excretion of bromide from the body, which could be demonstrated, was via the kidneys. Excretion of bromide by the feces was extremely low.

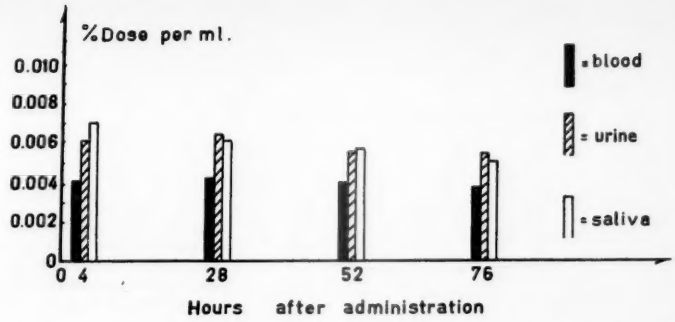

Fig. 1. The average percentage of radio-bromide of the total given dose per ml blood, urine, and saliva per day.

Material and Methods

Thirteen students, five women and eight men, were selected on characteristics of good general health. They engaged in normal duties and enjoyed a diet normally varied. The age was 21 years on an average. The weight and height of the subjects are tabulated in Table I.

The subjects were orally given 10 μC Br^{82}-labelled ammonium bromide dissolved in 25 ml water. This corresponded to less 0.05 mg ammonium bromide.

Urine specimens were collected about every second hour from around 7^h to around 23^h. The importance of emptying the bladder at the end of each collection period and avoiding loss of urine was pointed out to the subjects. The volume of the excreted urine was measured at each collection period. In this way all urine excreted for four days was collected. From each of the specimens 5.00 ml urine was taken for measurement of the radioactivity. The volume multiplied by the activity per ml urine gave the total amount of Br^{82} in each specimen.

From six of the subjects, two women and four men, also saliva and blood samples were taken at various times after the administration. The saliva samples were obtained by letting the subjects chew paraffin wax. Roughly 6 ml saliva was collected about every fourth hour from about 9^h to about 21^h. Blood samples were drawn from the antecubital vein with heparinized syringes twice a day, 11.30 and 15.30 every day for four days.

The amount of Br^{82} in the samples of urine, blood, and saliva were measured with a well-type scintillation crystal and a Tracerlab autoscaler.

Results

About three hours after the radioactive solution was swallowed (by this time the steady state had been reached) it was found that the concentration of Br^{82} in the blood was on an average 0.0042 % of the total given amount of Br^{82} in every ml of blood. The concentration then decreased in an exponential form. The biological half-life of bromide ions was calculated and found to average 12 days for the six subjects.

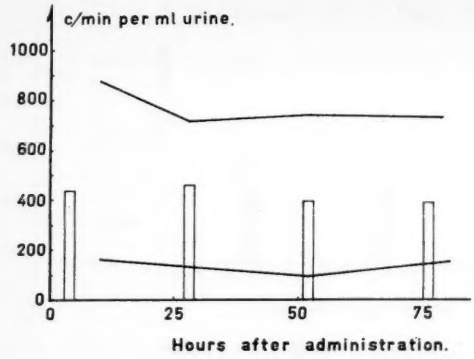

Fig. 2. The average relative amount of Br^{82} (counts per min per ml) in urine per day (mean from thirteen subjects). The standard deviation is also plotted.

The concentration of Br^{82} in the saliva samples was about 150 % of that in the blood. Also in the saliva the biological half-life was found to be about 12 days.

The concentration of radio-bromide in the urine was about as high as in the saliva. The biological half-life of bromide ions in the urine was about the same as in the blood and the saliva as can be seen in Fig. 1. As the time required for half of the amount of the administered bromide to be eliminated from the blood by normal biological processes was 12 days and, as can be seen in Table I, the mean of the amount of bromide excreted per day was 4.3 % of the given

Table I. Percentage of the given dose of radio-bromide excreted by the urine per day

Sex	Weight kg	Height cm	Days after an oral dose %				
			1	2	3	4	Mean
F	60	174	5.0	4.5	5.5	5.0	5.0
F	52	172	4.1	3.8	6.3	5.1	4.8
F	66	165	6.3	7.6	5.3	2.0	5.3
F	57	170	3.0	3.9	2.0	2.7	2.9
F	64	172	4.8	5.5	4.1	4.2	4.7
M	58	174	5.1	5.4	5.3	4.6	5.1
M	68	179	4.3	4.1	2.9	3.6	3.7
M	74	179	4.5	5.8	4.1	5.3	3.8
M	53	173	2.1	2.0	2.6	2.1	2.2
M	68	165	4.5	4.3	6.6	7.0	5.6
M	77	182	5.6	4.4	3.8	4.4	4.6
M	86	185	2.7	2.3	4.0	4.4	3.4
M	63	187	3.5	4.9	5.4	2.8	4.2

Mean excretion per day 4.3 % ± 1.0

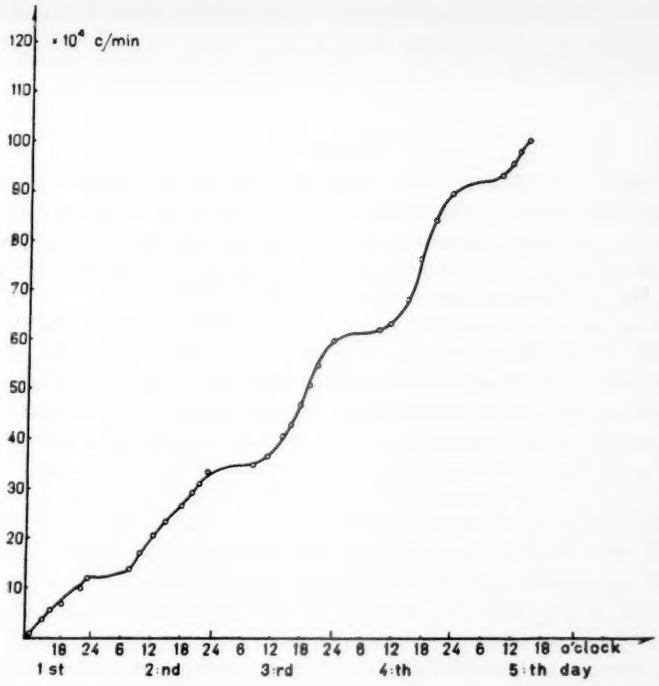

Fig. 3. The cumulative urinary loss of bromide. The curve represents the results with one of the subjects and is typical for all of the thirteen students investigated. Note, that there is no excretion of bromide during the subject's sleep.

amount Br^{82} it seems as the main way of excretion of bromide is by the urine. Consequently, all orally administered bromide was absorbed in the intestine.

It was found that the amount of bromide excreted by the urine varied extremely; see Table I and also Fig. 2, where the standard deviation is plotted.

The amount of radio-bromide excreted was highest between 11^h and 16^h. In the first urine specimen every morning there was in no case any significant amount of Br^{82} detectable. The cumulative excretion of bromide in the urine showed that during the night very small amounts of bromide were excreted; this fact is represented by the plateaus in Fig. 3. The longer the subjects slept the longer was the plateau.

In six of the subjects the exhalation gas of the lungs was allowed to pass through two solutions mounted in a series. The two solutions consisted of 10 ml of 4 N Na_2SO_3 and 0.2 N NaOH. Saliva was prevented to contaminate the

solutions by the use of drop-collectors. All the exhalation gas was collected for half an hour on each occasion, twice a day during two days. No significant amounts of radio-bromide could be detected in the solutions.

Discussion

BODANSKY and MODELL (1941) demonstrated that the amount of bromide excreted was related to the halide concentration of the experimental animal and also to the bromide/halide ratio in the plasma. The urinary loss of bromide was extremely divergent (SÖREMARK 1960) in spite of the fact that the animals were kept on identical diets.

It would seem as though the main way of bromide excretion is by the urine, and this is indicated by the fact that the present investigation showed a biological half-life of bromide in blood of 12 days and a daily loss of bromide by the urine of 4.3 % after a single dose of a trace amount of Br^{82} isotopes.

Excretion of small amounts of bromide may perhaps also take place via the skin, the nasal and conjunctival secretions as well as via the saliva.

It seems to be a point of interest that the present investigation showed a rhythmic excretion of bromide by the urine, this was clearly observable in all the subjects. The phenomenon of no loss of bromide by the urine during the nights was, however, not present in three subjects which were not allowed to sleep one of the nights. In these three cases there were no plateaus in the cumulative curves for those nights when the subjects did not sleep. It has previously been reported that there is a diurnal rhythm in the excretion of sodium and chloride (NOBLE 1957). As far as known this rhythmic excretion of bromide due to the sleep has not previously been reported.

It is known (cf. GOODMAN and GILMAN 1955) that bromide has as one of its pharmacological effects a depressing result on the central nervous system. However, it can be said that the physiological rôle of bromide in the intermediate metabolism has by no means been made clear (UNDERWOOD 1956). An explanation of the diurnal variation in the urinary loss of bromide from the body is therefore at present difficult to give.

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Acta physiol. scand. 1960. 50. 311—327

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Vasomotor Reflexes from Muscle

By

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Received 11 July 1960

Abstract

SKOGLUND, C. R. *Vasomotor reflexes from muscle.* Acta physiol. scand. 1960. *50.* 311—327. — Casual observations of blood pressure changes during muscle stretch drew attention to the possible role of muscle proprioceptors in vasomotor control. An attempt has been made to throw some light on this problem by studying the effects of mechanical muscle stimulation, as also of electrical excitation of muscle afferents, on systemic arterial pressure and on regional hindlimb circulation, in anesthetized or decerebrated cats.

Typical changes in systemic arterial pressure — *viz.* an initial depressor effect, followed or not by a more or less pronounced pressor effect — could be elicited by moderate stretch of different fore- and hindlimb muscles. The effects, which were dependent on intact nerve connections, appeared at tendon loadings of 100—300 g and could also be produced by slight pressure on the muscle belly or tendon. The conclusion that these effects were reflex responses mediated from mechanoreceptors in muscle was further substantiated by comparative experiments which, *i. a.*, showed the divergent responses to various types of painful stimulation applied to the muscle. For eliciting systemic depressor effects by electrical stimulation of muscle afferents, stimulus strengths of more than 10 times the threshold of group I fibers were required; this implies that no depressor response was observed until, after maximal activation of group I and II fibers, group III fibers were being excited.

By simultaneous recording of the local arterial pressure in the lower hindleg, perfused at constant rate, the occurrence of regional vasomotor effects — predominant vasodilatation or constriction — could be demonstrated during electrical or adequate activation of muscle afferents. Studies of blood flow in muscles by inserted thermocouples were also performed.

This investigation was initiated by accidental observations made during spinal cord experiments on cats to the effect that stretching of limb muscles may result in obvious changes in the systemic arterial pressure.

The earliest observation on vasomotor effects by mechanical stimulation of muscle seems to have been made — incidentally at this department — by KLEEN (1889) who, for the purpose of investigating the physiological effects of massage, kneaded exposed muscles of rabbit and found that this caused a typical lowering of the systemic pressure followed or not by a transient rise. That blood pressure changes occur on manipulation of muscles has since been confirmed by BRUNTON and TUNNICLIFFE (1894), HUNT (1895), VINCENT and CAMERON (1915), FLOREY and MARVIN (1928) and by VINCENT and THOMPSON (1928) who established the reflex nature of the depressor effect.

It is easy to understand that no further interest has been taken in these phenomena if they are considered only as non-specific vasomotor reactions, possibly enhanced by the anesthesia or by other abnormal conditions of an acute experiment. However, one cannot exclude the possibility that the blood pressure changes recorded during mechanical muscle stimulation reflect a physiologically significant mechanism, implying that muscle proprioceptors play a role as a peripheral link in the vasomotor regulation system. A closer analysis of the vasomotor effects observed during muscle stretch seemed therefore to be justified.

In the first section of this paper a general description of the systemic pressure changes occurring during mechanical stimulation of various muscles will be given. In the second section some comparative experiments on electrical stimulation of muscle afferents are described. In a third part of this work a first advance on the problem of the relation between the systemic changes and those of different vascular areas has been made by studying regional vasomotor effects in the hindlimb during adequate and electrical stimulation of muscle afferents.

Previous work relevant to sections II and III will be dealt with separately at the beginnings of these sections.

Methods

Most experiments were performed on cats narcotized with nembutal, sometimes with addition of thiogenal, or with chloralose-urethan; a few comparative experiments were also made on decerebrate cats. For curarization flaxedil was usually preferred to tubocurarine because of its less pronounced effects on the blood pressure.

In one series of experiments the tendons of the various muscles investigated were freed and attached to threads to which tension could be applied, either manually via a spring balance for measuring the applied force, or by application of weights. A device for marking onset and end of stretch on the record was as a rule in use. The legs were fixed with pins through the bones; cats laminectomized for dorsal root recording were also supported by pins in the spinal column. Removal of the skin over the muscles was sometimes performed in order to allow direct manipulation of the muscles without skin stimulation.

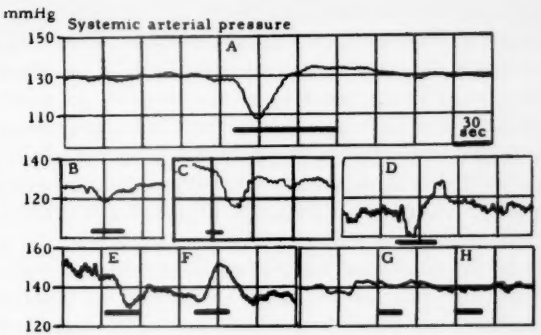

Fig. 1. Changes in systemic arterial pressure produced by stretching different fore- and hindlimb muscles. *A*, predominant depressor response of maximum amplitude; triceps surae. *B*, smaller depressor response; quadriceps. *C*, depressor response to light lateral pressure on muscle belly; gastrocnemius. *D*, depressor-pressor response; ext. dig. long. *E*, depressor response; gastrocnemius. *F*, pressor response; tib. ant. *G* and *H* same as *E* and *F* after section of sciatic nerve. Periods of stretch (or electrical stimulation) marked by solid black lines in this and following figures.

In another series of experiments the operative procedure was limited as far as possible in order to exclude painful stimulation, as further described in section I.

The systemic pressure was measured in the carotid artery. The level changes in the mercury manometer were recorded by a capacitance meter on a Siemens inkwriter, the slow time constant of which (1 sec) only allowed recording of a mean arterial pressure.

In some experiments the lower hindleg was perfused via the femoral artery by means of an adjustable flow-rate pump connected to a polyethylene loop in the femoral artery and allowing constant perfusion rates between 15 and 50 ml per minute. The regional pressure peripheral to the pump was recorded via a Statham pressure transducer on a second channel of the Siemens inkwriter. Since the flow was kept constant during an experimental period, variations of this pressure indicate changes in peripheral resistance.

Regional vasomotor effects were also studied by recording the muscle temperature by unheated thermocouples inserted into the muscles. The use of a Hewlett-Packard chopper amplifier allowed readings of temperature variations of 0.01° C; for the recording another Siemens inkwriter was used.

Square wave stimuli of a frequency of 10/sec and of 0.1 or 0.5 msec duration were applied in the series of experiments on electrical stimulation of muscle afferents. Dorsal root recording was performed by use of conventional amplifier technique, sometimes in combination with an electric device for derivation of the action potential, which was found to be useful for identification of different components of the potential.

Results

1. Changes in systemic arterial pressure by muscle stretch

General description. The changes in systemic arterial pressure obtained by stretching a muscle may vary somewhat with factors such as the depth of anesthesia, the general condition of the preparation, the prevailing systemic pressure etc. However, the initial effect most commonly observed was a lowering of the pressure, a typical example of which is given in Fig. 1 *A*. The depressor

effect reached its maximum in 10—20 sec and then usually subsided in spite of maintained stretch, the average duration of the whole change lasting about 1/2 — 1 minute. In this case the maximum amplitude of the depressor effect amounted to 20 mm Hg; in less excitable preparations it might be smaller than 10 mm Hg even when larger muscles were stretched (Fig. 1 *B*).

The depressor effect may be followed by a phase of increased pressure which, as in *A* and *B*, is usually very little marked. In other cases the subsequent pressor effect had an amplitude equally large as or even larger than the initial depressor effect, as illustrated in *D*.

It was a general finding in anesthetized animals that by light stretch a depressor response could be obtained from practically every muscle tested on fore- or hindlegs; in decerebrate cats pressor responses occurred more often even at threshold stimuli; in other respects no essential differences were observed with these preparations. Strong pulling at the tendon could elicit pressor effects in all types of preparations. The possible interference of nociceptive stimulation in the latter type of response will be discussed below.

However, at similar amounts of stretch striking differences were sometimes observed between different muscles in one and the same experiment, as illustrated in *E* and *F*. In marked contrast to the typical depressor effect in *E*, obtained by stretching gastrocnemius, is the response to stretch of tibialis anticus at *F*, where the initial depressor effect is abortive and the pressure effect predominant. Although experiences from more than 25 cats showed that pressor effects were more easily elicited from flexors than from extensors at the ankle it is not possible, judging from experiments on other muscles on fore- and hindlegs, to relate the difference to functionally different muscle groups. However, whatever phase is dominating, the effects are dependent on an intact nerve supply, as seen by comparing *E* — *F* with *G* — *H*, and hence represent reflex responses. The interesting question of the relation between depressor and pressor responses — whether they are dependent on quantitative or qualitative differences in afferent inflow from muscle or result from predetermined central innervation patterns — will not be considered in the present analysis which is mainly limited to depressor effects.

The vasomotor reflexes remained after curarization, the depressor effects often becoming more pronounced and constant; in lightly anesthetized, non-curarized cats, showing extensive reflex movements in response to muscle stretch, complex sequences of depressor-pressor effects appeared which were difficult to analyze.

Control experiments have shown that changes in respiration and heart rate have no significant part in the phenomena studied. Elimination of circulatory reflexes from sinus caroticus did not alter the general picture of pressure changes.

Variations in the experimental procedure. In order to determine the possible influence of unintentional nociceptive stimulation, several variations in the

experimental procedure were tried. Thus, in some experiments local injection of novocaine into the tendon was made in order to prevent afferent inflow of pain impulses from this region; typical depressor effects were still obtained, as also pressor effects. However, it cannot be excluded that, under standard experimental conditions, pain impulses from the tendon region may contribute to the pressor effect, especially at strong pulling, since pain stimulation applied on purpose, *e. g.* by pinching the tendon with a forceps, was invariably observed to cause rise in blood pressure (cf. also section III).

One series of experiments was performed in which the leg was not fixed by drill-pins but kept in position manually; the typical vasomotor effects by pulling at the tendon could be reproduced also under these conditions. When fixation pins were inserted, blood pressure changes could be produced by exerting pressure on the pins or by touching the operative wounds, but the degree of dislocation required was such that it was not likely to occur during the standard experimental conditions; the effects obtained were always of pressor type (cf. EULER and SJÖSTRAND 1936).

Some experiments were made on completely intact legs, and even under these conditions blood pressure variations could be observed when passive extension or flexion movements were performed at different joints. Naturally an experimental situation like this involves many uncontrolled factors such as variations in the afferent inflow from joint and skin receptors, and the interpretation of the results may not be simple. One typical experiment will, however, be mentioned: characteristic depressor effects were obtained by extension movements at the knee-joint and in this case it could be shown that the dominating afferent inflow responsible for the effect came from the flexor muscle of the thigh, since the vasomotor effects could no longer be obtained when the distal insertions of the muscle had been dissected free from the tibia. By applying stretch to the freed end of the muscle the effects could again be reproduced.

In this experiment, as in many others, KLEEN's original finding that kneading of a muscle may give depressor effects could be confirmed. Only light pressure on the muscle belly was sometimes enough to elicit a typical depressor response which, as appears from Fig. 1 *C*, is very similar to the effect obtained by stretch. Under such conditions changes in local circulation shown to occur with more forceful kneading (BRUNTON and TUNNICLIFFE 1894) can certainly be excluded as causes of the observed effects. It was also often observed that slight pressure in the tendon region was an effective stimulus for eliciting depressor effects.

Threshold determinations. In some ten cat experiments quantitative determinations of the increase in tension necessary to produce the characteristic depressor effects were performed. A typical experiment is illustrated in Fig. 2 *A*. It appears that a tension of 100 g was subthreshold, while 125 g gave a just visible effect. 150 g caused a somewhat larger and more prolonged effect, and with 250 g tension a maximal lowering of about 20 mm Hg was obtained. At this

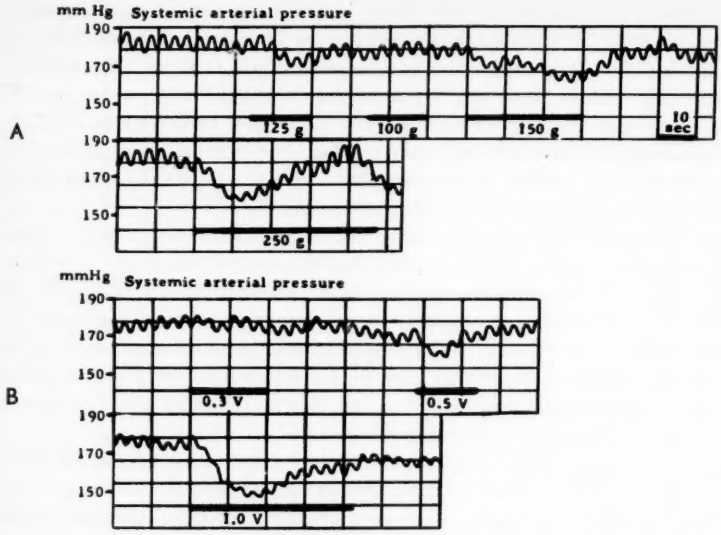

Fig. 2. Systemic pressure changes produced by *A*, graded stretch of triceps surae, and *B*, graded afferent stimulation of the biceps-semitendinosus nerve. Applied tensions and stimulus strengths as indicated by figures below stimulus markings. Stimulus duration 0.5 msec.

loading there was a tendency to a subsequent pressor effect, illustrating the previously mentioned appearance of pressor responses when strong pulling at the tendon was exerted. It should be remarked that this type of experiments allows only of an approximate estimation of thresholds, since these may vary with several factors, among which is the rate of stretch, which was not controlled in these experiments. The example chosen represents an average result; threshold values as low as 50 g have only occasionally been observed, while in other experiments 200 — 300 g have been necessary for just observable responses. The limited material does not allow of any definite conclusion as to differences between various muscles.

II. Changes in systemic pressure by electrical stimulation of muscle afferents

Changes in systemic arterial pressure by afferent stimulation of pure muscle nerves were described by Asp (1867) who found both pressor and depressor responses in rabbits, while Tengwall (1895), using the same animal, came to the conclusion that muscle nerve stimulation always resulted in depressor effects provided current escape to skin nerves was avoided[1].

[1] In a second section of the same paper, Tengwall, at that time a medical student at Karolinska institutet, made an early contribution to mammalian reflex physiology by demonstrating the powerful action of muscle afferents in eliciting muscle reflexes!

In the numerous studies of vasomotor effects by afferent nerve stimulation performed since that time, mixed or cutaneous nerves have mostly been used (cf. GORDON 1943, FERNANDEZ DE MOLINA *et al.* 1953, LAPORTE and MONTASTRUC 1957, in which papers references to the older literature will also be found). However, since many of the mixed nerves used in these studies consist chiefly of muscle afferents, it is safe to assume that the depressor responses to mixed nerve stimulation are to a large extent to be ascribed to activity in muscle afferents. The types of muscle afferent fibers involved may in fact be predicted from some negative findings in these investigations. Thus, FERNANDEZ DE MOLINA *et al.* (1953) found that the fastest fibers of the A group were not essential for vasomotor effects of depressor type, and this excludes the group I band of muscle afferents. Furthermore, MARTIN and LACEY (1914) had found that the thresholds for depressor effects were somewhat higher than those for flexor reflexes, which are now known to be mediated by group II or group III fibers (LLOYD 1943).

It seemed, however, desirable to get more direct evidence of the types of fibers concerned, by means of a reinvestigation, using modern electrophysiological techniques, of vasomotor effects caused by stimulation of pure muscle nerves. After this had been accomplished (cf. SKOGLUND 1959), confirming the predicted ineffectiveness of group I fibers in eliciting typical depressor effects for which activation of group III fibers was required, results from a similar, more extensive investigation have been published by LAPORTE, BESSOU and BOUISSET (1959, 1960). These authors have come to the same conclusions and, in addition, presented detailed information about the appearance of pressor and depressor effects under varying experimental conditions. The publication of their data has made it possible to restrict the description of results in this section to the data relevant to the special problem of this investigation.

Previous investigations on mixed nerves had shown that the blood pressure effects are dependent, besides on strength, also on the frequency and duration of the stimulus, and it could *a priori* be assumed that the same applies to stimulation of pure muscle nerves. On the basis of a limited study of the effects of variation of the stimulus parameters, and in view of previous data indicating that short stimulus duration and low stimulus frequency favor depressor responses (GORDON 1943), a stimulus frequency of 10 per sec and stimulus durations of 0.1 and 0.5 msec were chosen as standard conditions for the present analysis, which is mainly limited to studies of depressor effects.

The typical effects on systemic pressure by such a stimulation are illustrated in Fig. 2 *B*. It appears that the depressor responses, which increased with the stimulus strength within certain limits, may be very similar to those obtained in the same experiment by muscle stretch, Fig. 2 *A*. Thus, both the initial rate of change and the maximum amplitude are about the same. Adaptation of the response takes place in both cases, but with strong artificial stimula-

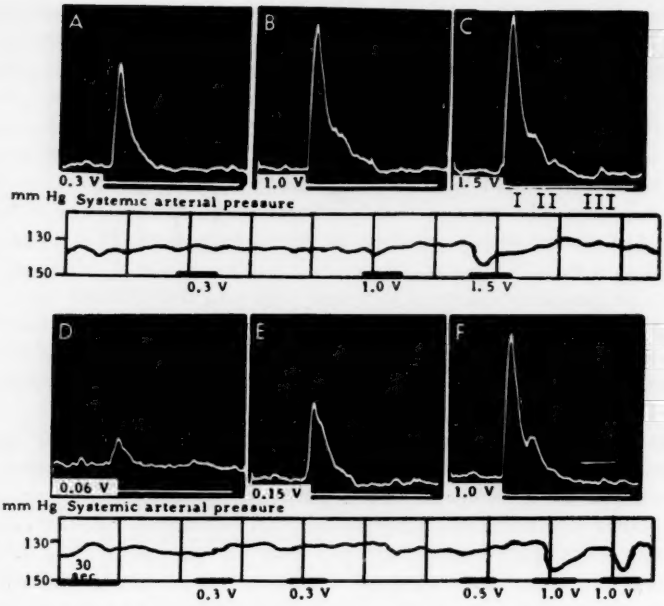

Fig. 3. Systemic depressor effects in relation to afferent volleys in the biceps-semitendinosus nerve recorded from small dorsal root filament. *A* — *C*, 0.1 msec and *D* — *F*, 0.5 msec stimulus duration. Stimulus strengths indicated by figures in each potential record and below stimulus markings in the blood pressure recordings. Potential peaks of group I—III fibers marked in *C* (group III spike clearly visible only at higher gain). Time bar in *F* 1 msec.

tion (at 1.0 V) a full return to the initial pressure does not occur during the stimulation period and the depressor effect may also remain for a considerable time after the end of the stimulation. The faster adaptation with natural stimulation is most likely due to an adaptation at receptor level. It can be seen that a stimulus strength of 0.3 V did not give any clear effect, while a distinct depressor response is obtained by 0.5 V. Maximum effect is attained at 1.0 V. The record gives an idea of the degree of accuracy with which the threshold can be determined. In this case it was estimated at 0.4 V, representing a stimulus strength ten times the threshold for group I fibers.

This ratio was determined in the following way. A small dorsal root filament containing afferent fibers from the muscle nerve stimulated was sectioned and placed on recording electrodes so that the afferent volley in this filament could be recorded simultaneously with the vasomotor effects due to activity in the intact fibers. Typical records thus obtained are shown in Fig. 3, from another experiment where two series of experiments with different stimulus duration had been performed. From the upper series of records, where a

stimulus duration of 0.1 msec was used, it appears that a maximum group I spike could be attained and also group II fibers activated (at 1.0 V) without any systemic depressor effects being visible. A distinct depressor effect was obtained only when the stimulus strength was increased to 1.5 V. The threshold of group I fibers was 0.1 V. The blood pressure effect was thus not attained until the stimulus was increased 15 times the threshold.

In the lower series of records, where a longer stimulus duration, 0.5 msec, was used, the absolute threshold values are typically lower, the threshold for group I fiber activation being estimated at 0.05 V and that for the depressor effect at about 0.6 V. The ratio was 12, thus of the same size of order as with the shorter stimulus duration.

Analysis of the action potentials showed that, on an average, maximum group I fiber activation of the biceps-semitendinosus nerve was reached at 4—5 times the threshold. Group II fibers were activated at about twice the threshold, and their maximum activation was estimated at 8—10 times the threshold for group I fibers. At about the same multiple of threshold group III fibers were activated. These values are higher than those observed by Brock *et al.* (1951) but correspond quite well to those given for the gastrocnemius nerves by Eccles and Lundberg (1959). The analysis thus showed that distinct depressor effects appear simultaneously with activation of group III fibers but a participation of high-threshold group II fibers cannot be entirely excluded.

With careful nerve preparation, the absolute values of stimulus thresholds varied surprisingly little in different experiments for a given stimulus duration. In ten experiments on different cats, the range of the threshold values for vasomotor effects was 0.4—0.8 V (mean value 0.6 V) for 0.5 msec duration and 0.8—1.5 V (mean value 1.0 V) for 0.1 msec stimulus duration. The corresponding mean values for group I fiber activation were 0.05 and 0.1 V. The average ratio between the different threshold values was thus about 10.

III. Changes in regional circulation by electrical stimulation of muscle afferents and by muscle stretch

After Lovén's (1866) demonstration of regional vasomotor responses to stimulation of afferent nerves, a great number of such investigations have been carried out. One of the earliest was made by Gaskell (1878) who showed that stimulation of mixed afferent nerves caused an increased venous outflow from muscles, indicating a dilatation of the muscle arteries. This effect was shown to occur at stimulus strengths causing an actual or "potential" (curare-blocked) reflex contraction in ipsilateral flexor muscles. However, stimulation of pure muscle nerves does not seem to have been tried in this type of experiments. The only observation on vasomotor changes during muscle stretch seems to be one by Denny-Brown (1929) who, by watching the cat's soleus muscle under microscope during active tension of a stretch reflex, found that an increase

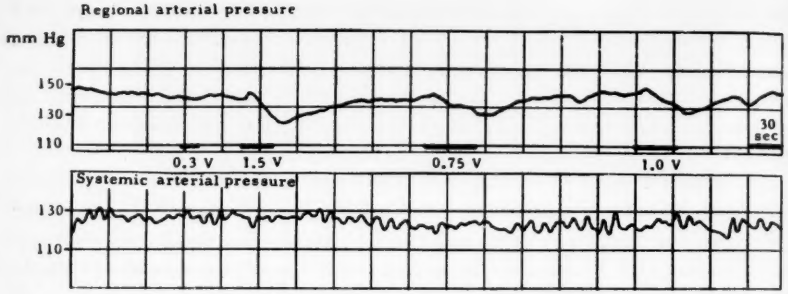

Fig. 4. Lowering of regional arterial pressure of perfused lower hindleg caused by stimulation of the ipsilateral biceps-semitendinosus muscle afferents. Strengths of stimuli applied to the nerve indicated by figures below stimulus markings.

in blood flow occurred in the capillaries of the muscle surface as well as in somewhat deeper vessels.

From previous work on local circulation in different vascular areas it is obvious that the analysis of regional vasomotor effects may be complex and require various advanced techniques, which by themselves may influence the physiological events studied. A full investigation of reflexly induced vasomotor changes in muscle is also certainly a large project, comparable to analysis of motor reflexes. The aim of the present approach to this problem was, in the first line, only to establish whether changes in regional limb circulation occur in response to activity in muscle afferents. A comparison between regional and systemic effects might also throw some light on the nature of the systemic pressure change.

One type of experiments, in which perfusion technique was applied, served to give information of gross changes occurring within the vascular area of the whole lower leg. The perfused region is not homogeneous; vascular beds of muscles dominate but also skin vessels contribute, since even after removal of the skin over the lower leg the richly vascularized pad area remains. However, typical changes in the regional pressure of the perfused area could be observed when muscle afferents on the ipsilateral side were stimulated. As shown in Fig. 4, stimulation of the biceps-semitendinosus nerve resulted in a lowering of the regional pressure, indicating a decrease in resistance to flow, which in its turn shows that vasodilatation predominates within the vascular region perfused. In this case the distinct changes made it possible to determine the thresholds rather accurately. The effect at a stimulus strength of 0.75 V was somewhat larger than the smallest distinguishable effect at 0.6 V (not illustrated). It can be seen that both the amplitude and the rate of change of the depressor effect increased with increasing stimulus strengths up to a certain value, 1.5 V.

The absence of distinct systemic depressor effects — the influence on general circulation being mainly indicated by a disturbance of the rhythm of Trauber-

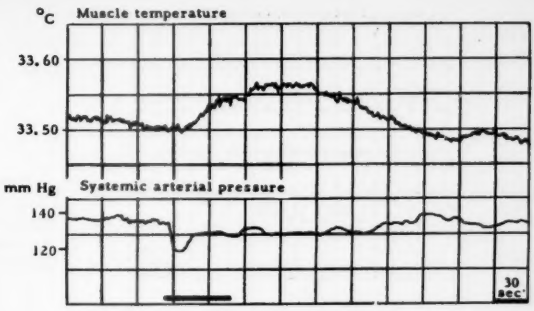

Fig. 5. Increase of blood flow in one muscle (tibialis anticus) and lowering of systemic arterial pressure caused by stimulation of muscle afferents of another muscle (gastrocnemius) on the hindleg. Blood flow measured as temperature change by inserted thermocouple.

Hering's waves — is by no means characteristic of the perfusion experiments but is an example of a decreased excitability sometimes observed at later stages of any experiments. It appears that the threshold value in the illustrated experiment corresponds to the average value in the series of systemic pressure experiments, and as a rule the threshold for regional circulation changes was the same as for systemic effects. In this type of experiments dorsal root potentials have so far not been recorded and the relation to group I fiber thresholds could therefore not be determined, but it may safely be concluded from the high absolute threshold values alone that more high-threshold than group I fibers are involved.

Regional circulation changes in response to electrical stimulation of muscle afferents were also investigated by measuring the muscle temperature by means of inserted thermocouples. This method has the advantage of causing a minimum of disturbance of the physiological conditions, but its value is limited by the fact that artifacts are easily produced by movements of muscle tissue in relation to the thermocouple. The method can therefore not be applied to the muscle under stretch, and full curarization is necessary when vasomotor effects in reflexly activated muscles are being studied. Under the prevailing experimental conditions, with the muscle kept at subnormal temperature, a temperature rise indicates an increase in blood flow, but it should be observed that only in combination with an unchanged or lowered systemic pressure can this effect be interpreted as due to a local change in vasomotor tone, since an increase in systemic pressure may be accompanied by a passive increase in blood flow.

A favorable situation for analysis exists, however, in the experiment illustrated in Fig. 5, where the effects of afferent stimulation of the gastrocnemius nerve were studied. Simultaneously with the typical lowering of the systemic pressure there occurs a rise in temperature of the studied muscle (tibialis ant.), which may thus be referred to an increase in flow due to regional vasodilatation. The absolute threshold value was the same as for the systemic effect and typically high, 1.5 V, indicating activation of higher threshold muscle afferents.

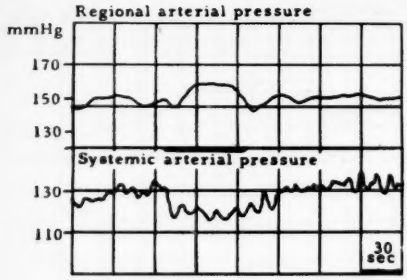

Fig. 6. Increase in regional arterial pressure of perfused lower hindleg (largely mechanical effect) and lowering of the systemic pressure caused by stretch of ipsilateral triceps surae.

Attempts were made to record temperature effects in the same muscle from which the afferents arise, by stimulating one branch of the gastrocnemius nerve, leaving the other intact for mediation of vasomotor responses. However, the effects proved to be rather poor, possibly due to insufficient reflex activation under these conditions, and allowed of no definite conclusions whether the thresholds in this case were lower.

In some experiments temperature changes were recorded from two different muscles simultaneously, and in certain cases reciprocal effects were noted, *e. g.* in muscles on both sides, but because of the interpretation difficulties mentioned above (one of the contrary effects may always be passive) no conclusions can be drawn from these experiments until complementary methods of analysis have been applied (cf. below). It might be mentioned in this connection that in a study of vasomotor effects by exteroceptive stimulation (ÅSTRÖM and SKOGLUND, unpublished results) contrary effects on regional arterial pressure of one hindlimb could be demonstrated when nociceptive stimulation was applied to the pads on the two sides.

It was *a priori* to be expected that studies of regional pressure changes caused by stretching a muscle within the perfused area might be complicated by purely mechanical effects on circulation. In the experiment of Fig. 6, stretch was applied to the tendon of triceps surae. Parallel with the typical lowering of the systemic pressure there occurred a rise in the regional pressure. It could be shown that this effect was to a large part mechanical, since it was only slightly reduced by section of the motor nerve. It is obvious that regional vasomotor changes of reflex nature are easily concealed by such mechanical effects. However, this type of experiment may serve to illustrate that the systemic depressor effect due to stretch appears in spite of a maintained or even raised pressure in the regional limb circulation.

When the muscle subjected to stretch is outside the perfused region these experimental difficulties do of course not exist. Fig. 7 illustrates the pressure changes that may occur in the perfused left hindleg when the right triceps surae was repeatedly stretched. The curve indicates that a regional vasoconstriction takes place parallel with the lowering of the systemic arterial pressure.

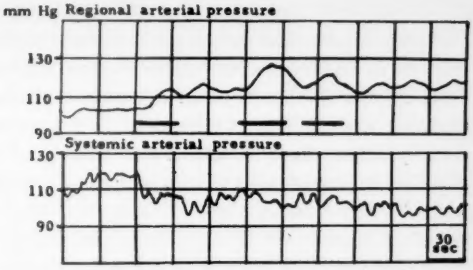

Fig. 7. Increase in regional arterial pressure of perfused lower hindleg and lowering of systemic pressure caused by stretch of the contralateral triceps surae.

It is obvious that further studies of regional effects in more restricted areas, preferably by perfusing individual muscles, will be necessary to give information of the detailed pattern of vasoconstriction and vasodilatation changes which apparently take place during changes in the afferent inflow from muscles.

In this section will finally be included an example from a comparative series of experiments performed to illustrate the difference between mechanical and nociceptive muscle stimulation. It has already been mentioned that nociceptive stimulation by pinching the tendon or the muscle belly always causes a rise in the systemic blood pressure. Except pinching, which may involve mechanical stimulation, also another method of stimulation has been tried, *viz.* injections of hypo- or hypertonic solutions which from human experiments are known to cause pain (cf. LEWIS 1942). 6 per cent NaCl solutions have been tested, and even when injected in small amounts of a few tenths of a ml these have a very marked pressor effect, but also hypotonic solutions may cause a typically long-lasting elevation of the systemic arterial pressure (Fig. 8). As can be seen, the regional pressure is lowered simultaneously, indicating a regional vasodilatation in the perfused area. These records may serve as another example of how the systemic and regional vasomotor effects during activation of muscle afferents may vary in different directions.

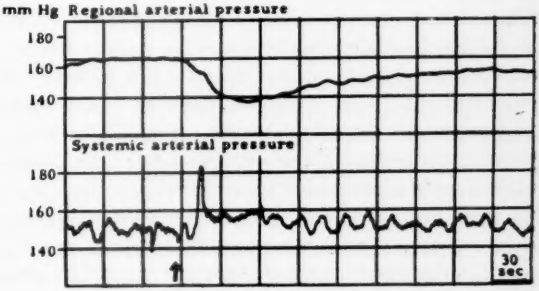

Fig. 8. Lowering of regional arterial pressure and increase of systemic pressure caused by nociceptive stimulation (injection of 0.2 ml distilled water in biceps brachii at arrow).

Discussion

The previous description has illustrated the stimulus conditions under which *changes in systemic pressure* occur during mechanical stimulation of muscle as well as electrical activation of muscle afferents, but whether the recorded changes are physiologically significant phenomena or not cannot be judged simply from these results. Narcotized and decerebrate preparations may display, in exaggerated form, reactions which are not seen in the intact animal. On the other hand, the fact that the blood pressure of the normal animal does not vary in the manner described during changes in afferent inflow from a muscle need not imply that the phenomena observed in the experiments are unphysiological. A peripheral mechanism providing the vasomotor centers with afferent impulses from muscles may still be functioning, but its reflex effect may be suppressed by counteracting influences from other sources. As long as this possibility has not been ruled out, it seems justified to pay some attention to this type of systemic pressure changes. The study of these changes has also led to the demonstration of regional vasomotor effects from muscle, the functional significance of which is more obvious. The experiments carried out so far have shown that the systemic effects are not a result only of local vascular changes occurring in one hindlimb. Recordings of regional vasomotor changes in different vascular areas are evidently required for elucidating the mechanisms of the systemic pressure changes.

The question of which receptors are involved in the systemic effects induced by mechanical stimulation of muscle may first be discussed. From HUNT's (1954) work it is known that the threshold for activation of muscle spindles by stretch is relatively low, *i. e.* about 3 g for firing of group I A fibers and 20 g for discharges in group II fibers, while the tendon organs require a tension of 100—200 g or more for sustained firing. This would suggest that in the first place tendon organs might be responsible for the vasomotor effects recorded in these experiments, where a threshold below 100 g has only exceptionally been observed. However, a high threshold for muscle stretch need not exclude a participation of muscle spindles, since it is possible that a more massive afferent inflow, such as occurs at heavier loading, is necessary to overcome the "central threshold" and produce an observable reflex effect. On the other hand, if the vasomotor centers generally require a more intense bombardment it is remarkable that such a small stimulus as light pressure on the muscle belly may sometimes produce large changes in systemic pressure. One explanation might be that in the latter case not only muscle spindles but also other, specific receptors with more direct and effective central connections with vasomotor centers are activated. Various types of encapsulated end organs and free endings described in muscle (cf. TIEGS 1953) have to be taken into account. Unfortunately only little is known about the physiological properties of these receptor types. It is of interest, however, that KOBAYASHI *et al.* (1952) have described slowly adapting impulse discharges in myelinated fibers below 7 μ, which were

elicited by pressure in the tendon region, as also in definite, restricted parts of the muscle belly. Stretch receptors in blood vessels (cf. AVIADO and SCHMIDT 1955) may also be activated during mechanical muscle stimulation.

The experiments with electrical stimulation of muscle afferents were made as an attempt to illuminate further the question of which afferent systems in muscle are engaged in vasomotor effects. It was found that in order to evoke systemic depressor effects a stimulus strength of at least 10 times the threshold of group I fibers was required, which implies that no response was observed until, after maximal activation of group I and possibly also group II fibers, group III fibers were excited. Since group I A fibers transmit impulses from primary endings of muscle spindles and group I B fibers from tendon organs (HUNT 1954), the results would indicate that discharges from these types of receptors would not be effective in eliciting depressor responses. It cannot be excluded that a maximal activation of group II fibers in some experiments may have contributed to the depressor effects, and this would indicate that secondary endings of the muscle spindles might be involved. It is of interest that LAPORTE *et al.* (1960) have demonstrated distinct pressor effects by stimulation of these fibers at a higher rate. The results of electrical stimulation are thus partly conflicting with the conclusions drawn from the experiments with stretch stimulation. However, the two types of experiments may not be directly comparable. That the evoked depressor effects are similar does not necessarily mean that identical afferent systems are activated during natural and electrical stimulation. A negative outcome of electrical stimulation may only mean that the impulse pattern set up is inadequate for central transmission. The effectiveness of high-threshold fiber stimulation suggests, however, that receptors connected with afferent fibers of smaller diameters are involved (cf. above).

In the experiments on *regional vasomotor effects* so far performed, the thresholds were equally high as for systemic effects. Since the effects were mostly studied in other muscles than that from which the afferent inflow originated, reflex contraction of the muscles also required stimulation of high-threshold fibers (group II or group III, LLOYD 1943). The results are thus in essential agreement with GASKELL's old finding (cited above) that vasodilatation in muscle due to afferent nerve stimulation occurs at the same stimulus strengths as cause contraction. Analysis of vasomotor effects in the same muscle from which the afferents arise, stretch reflex contraction of which is mediated by coarse fibers, will apparently constitute a key experiment for judging if vasomotor effects are always mediated by finer afferent fibers or not.

This brings up the problem whether the regional vasomotor effects elicited by muscle stretch are *primary*, due to specific receptor activity directly mediated to the vasomotor centers, or *secondary*, due to proprioceptor activity eliciting motor reflexes to which the vasomotor effects are automatically linked by pre-set couplings between higher motor and vasomotor centers. Comparative studies of regional vasomotor effects elicited from the periphery and from

higher centers may give a clue to this problem. Judging from other investigations (*e. g.* GREEN and HOFF 1937, cf. also FOLKOW 1960) it is very likely that the vascular changes during contraction are initiated from higher centers, and the muscle receptors may then have only a secondary role in local circulatory adjustments during phasic muscle contractions.

However, specific muscle receptors regulating vasomotor innervation may play a more significant role in tonic muscle contractions. The importance of the general tone of the muscles for circulation is well established by many investigations on man and animals, from which it is obvious that serious disturbances in general circulation may occur as a result of a decrease in muscle tone. Among the mechanisms in play for the regulation of vascular and muscular tone, attention may be drawn to the pressure receptors of sinus caroticus which regulate both vasomotor and muscular tone. As shown by SPYCHALA (1932), SCHWEITZER and WRIGHT (1937) and others, an increase in blood pressure will lead to a loss in muscle tone and *vice versa*. According to SCHULTE *et al.* (1959) this effect occurs mainly via the small motor nerves modulating the sensitivity of the muscle spindles, by which the stretch reflex is secondarily influenced. As recently shown by HUNT (1960), the muscle spindles are also under direct sympathetic control, and the threshold changes induced by sympathetic stimulation may even be imitated by adrenalin. In view of such mechanisms for central coordination of vasomotor and sympathetic tone, on one side, and muscle tone, on the other, it would seem appropriate with a peripheral receptor system in muscle feeding back information about muscular tone to the autonomic centers, and it is possible that the vasomotor phenomena observed during muscle stretch reflect such a mechanism.

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Observations on Central Regulation of Body Temperature and of Food and Water Intake in the Pigeon (Columba livia)

By

B. Åkerman, B. Andersson, E. Fabricius and L. Svensson

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Abstract

Åkerman, B., B. Andersson, E. Fabricius and L. Svensson. *Observations on central regulation of body temperature and of food and water intake in the pigeon (Columba livia).* Acta physiol. scand. 1960. *50.* 328—336. — Electrical stimulation within restricted parts of the hypothalamus and the preoptic area of the pigeon was found to elicit polypneic panting, hyperphagia and polydipsia. The spatial arrangement in the brain giving rise to the different effects corresponded well to that found in mammals. It may therefore be assumed that central regulation of body temperature, food and water intake is principally the same in birds and mammals.

The importance of the mammalian hypothalamus for the regulation of body temperature and of food and water intake has been elucidated by numerous investigations. The preoptic area has been shown to be the site of a "centre" regulating against hyperthermia (Magoun *et al.* 1938, Clark, Magoun and Ranson 1939). The lateral hypothalamus seems to be responsible for appetite (Anand and Brobeck 1951, Delgado and Anand 1953, Larsson 1954), whereas the ventromedial part of the hypothalamus apparently takes part in reactions of satiety (Brobeck, Tepperman and Long 1943). The urge to drink evidently emanates from an area of the hypothalamus located medial to the "appetite centre" (Andersson and McCann 1955 a and b). The role played by the avian hypothalamus and preoptic area in the

regulation of these functions is much less known. However, FELDMAN *et al.* (1957) have shown that aphagia can be produced by lesions in the posterior lateral hypothalamus of the chicken.

The present study was undertaken as an attempt to elucidate further the importance of the avian hypothalamus and preoptic area for the regulation of the above mentioned functions.

Methods

Forty-four fullgrown pigeons (Columba livia) of both sexes were used. Platinum iridium (Pt/Ir = 85/15) or nichrome electrodes (0.2 mm in diameter) with 0.1 to 0.2 mm uninsulated tips were implanted into the hypothalamus or neighbouring parts of the brain stem. The required length and the direction of the electrodes to reach the hypothalamus was judged from previous studies in dead material and from the experience gained during preliminary experiments. During the operation, the birds were placed in a special holder having their heads fixed by ear plugs and by a clamp holding the upper part of the bill. The scalp was anaesthetized by subcutaneous injection of xylocaine and an incision was made through the skin exposing the bone of the skull. Small holes were drilled through the bone and the electrodes were gently pushed downwards into the hypothalamus or adjacent areas. The bone sutures and the ear plugs were used as landmarks facilitating the correct placement of the electrodes. These were later fixed onto the skull bone by dental cement and the skin incision was sutured around the free ends of the electrodes. A metal wire fixed subcutaneously on the skull was used as an indifferent electrode. The bird was then placed in a cage where it was allowed to move freely during the experiment and the thin leads from the stimulator were soldered to the free ends of the electrodes. During the experiments the birds had free access to food (peas) and water. Some of the experiments were filmed.

Stimulation technique: The main characteristics of the stimulus used has been described earlier (ANDERSSON, PERSSON and STRÖM 1960 a). Mainly unipolar stimulation was applied. The resistance with the electrodes *in situ* was within the range of 7 to 10 kΩ. The parameters of stimulation were: Strength = 0.05 — 0.2 mA; Pulse width = 3 msec; Frequency = 50 cps.

On most occasions the experiments were completed on the day of the operation. The aminals were then sacrificed and the brains were fixed in formaline and later embedded in celloidine. Serial transverse sections, 50 microns thick, were made through that part of the brain which had been the site of the electrodes. The sections were stained with toluidin blue (Nissl) and according to the method of Loyez.

Results

A. Thermoregulatory response to stimulation.

Polypneic panting was obtained as an effect of electrical stimulation in 5 pigeons at sites located medially in the preoptic area somewhat anterior to and at the level of the anterior commissure (Fig. 1). At the two more posterior sites of stimulation (Fig. 1, c) the polypneic panting stopped immediately on cessation of stimulation. At the more anterior and ventral level (Fig. 1 a and b), in the medial and lateral preoptic nuclei, the polypneic response was seen

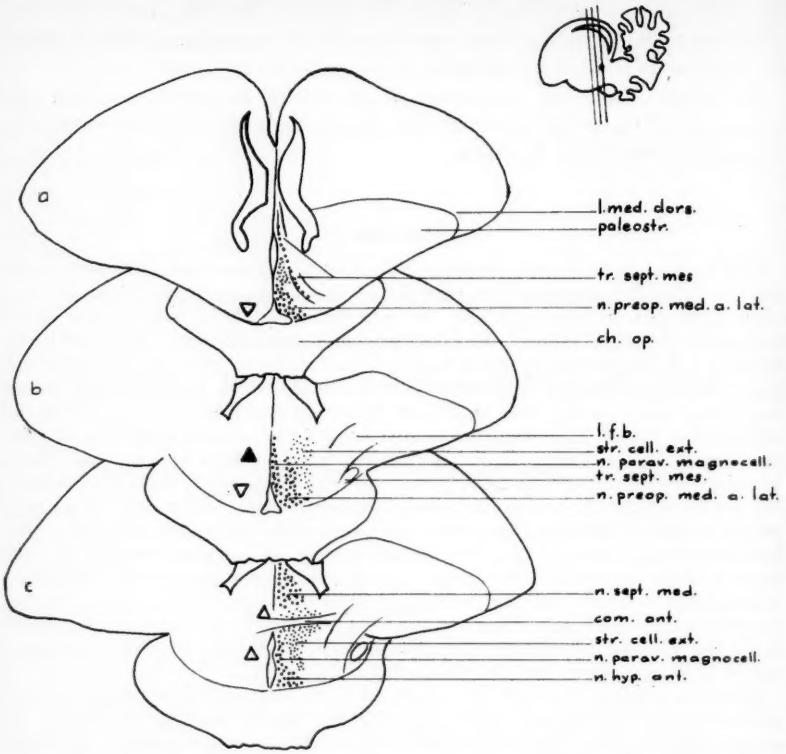

Fig. 1. Diagrams (× 8) of transverse sections of the pigeon brain showing the sites of stimulation at which thermo-regulatory response was obtained as effect of electrical stimulation. The level of the sections are seen up to the right.
▲ = Polypneic panting with after-effect.
△ = Polypneic panting without after-effect.
▽ = Polypneic panting starting immediately on cessation of stimulation.

Abbreviations:

a. vent. ant. = area ventralis anterior
ch. op. = chiasma opticum
com. ant. = commissura anterior
l. f. b. = lateral forebrain bundle
l. med. dors. = lamina medullaris dorsalis
n. hyp. ant. = nucleus hypothalamicus anterior
n. parav. magnocell. = nucleus paraventricularis magnocellularis
n. preop. lat. = nucleus preopticus lateralis
n. preop. med. = nucleus preopticus medialis
n. sept. med. = nucleus septalis medialis
n. sept. lat. = nucleus septalis lateralis
paleostr. = paleostriatum
str. cell. ext. = stratum cellulare externum
tr. oc. mes. = tractus occipito-mesencephalicus
tr. sept. mes. = tractus septo-mesencephalicus

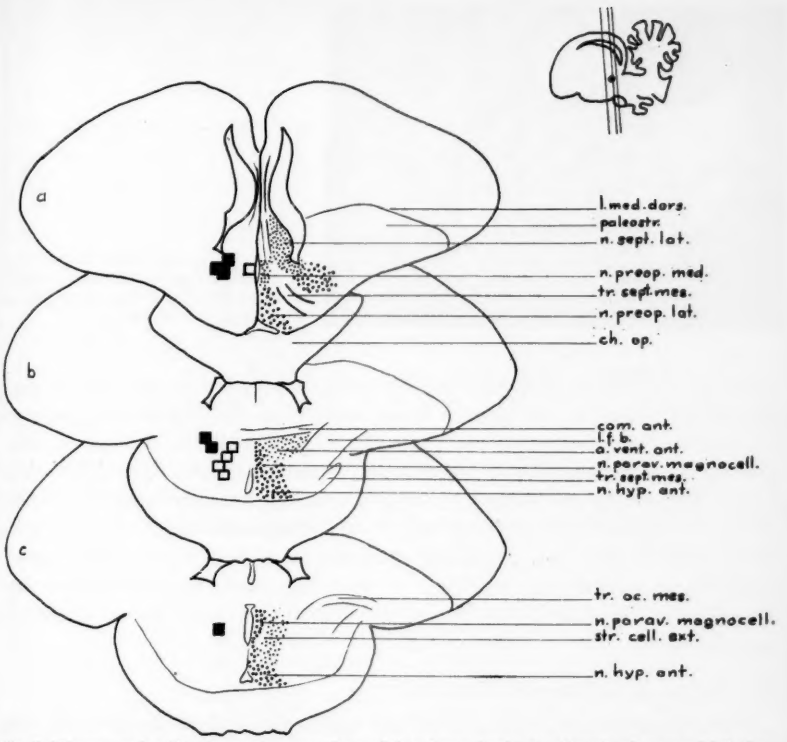

Fig. 2. Diagrams (× 8) of transverse sections of the pigeon brain showing the sites at which electrical stimulation caused hyperphagia and pecking. ■ = eating. □ = pecking. The level of the sections are seen up to the right. For abbreviations see Fig. 1.

to remain for up to about half a minute after the end of stimulation. The duration of the after-effect was seen to increase with increasing strength and duration of the electrical stimulation.

The polypneic response to stimulation seemed to be identical to that seen in the pigeon during hyperthermia. In some cases the panting was accompanied by a rather loud noise, consisting of a series of short sounds, apparently synchronized with the breathing rhythm. The identical noise was heard in other animals during hyperthermia caused by the heat from bulbs used for photographic recording.

B. Hyperphagia and pecking as effects of stimulation.

In 11 pigeons stimulations were seen to cause hyperphagia or pecking. The sites of stimulation were in the lateral hypothalamus and medial to the lateral forebrain bundle in the basal centres of the ventro-medial forebrain wall.

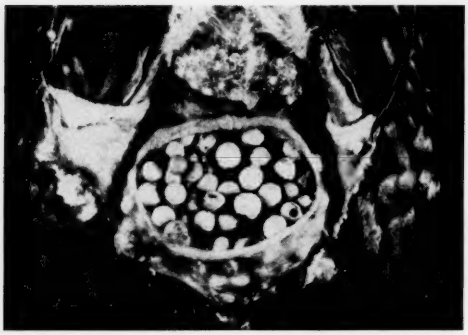

Fig. 3. The overfilled crop of one of the pigeons which showed considerable over-eating as result of prolonged stimulation.

Seen on transverse sections the sites of stimulation were at the level of and somewhat anterior to the anterior commissure (Fig. 2). The feeding response was most pronounced at the more lateral sites of stimulation and was usually preceeded by premasticatory movements of the mandibles. The birds then looked downwards and walked about in a searching manner, until they found the food container. The response was in some cases combined with circling in the ipsilateral direction. Prolonged stimulation in one of the birds caused considerable over-eating, resulting in vomiting when the stimulation was discontinued. Fig. 3 shows the crop of this animal filled with peas at the end of the experiment.

C. Polydipsia as a response to stimulation.

Stimulations in 7 pigeons close to the ventricular wall in the preoptic area and the anterior hypothalamus were observed to cause drinking. At the more medial sites drinking appeared during the period of stimulation. On prolonged stimulations in the nucleus preopticus lateralis and stratum cellulare externum, lateral to the nucleus paraventricularis magnocellularis, drinking was on the other hand obtained as an after-effect immediately on cessation of the electrical stimulation. In these cases the birds showed signs of restlessness during the actual stimulation. Drinking in some cases occurred together with bathing. The polydipsic response was typical of the drinking pattern normally seen in the pigeon. It can be described as a sucking motion keeping the bill inserted in the water.

The different sites at which drinking was obtained as an effect of stimulation are plotted in Fig. 4.

D. Other effects of hypothalamic and preoptic stimulation.

Stimulation in the hypothalamus and preoptic area also elicited many other responses, such as bathing, movements of the mandibulae, defecation, vomiting, alarm call, escape behaviour or defensive fighting against other pigeons or

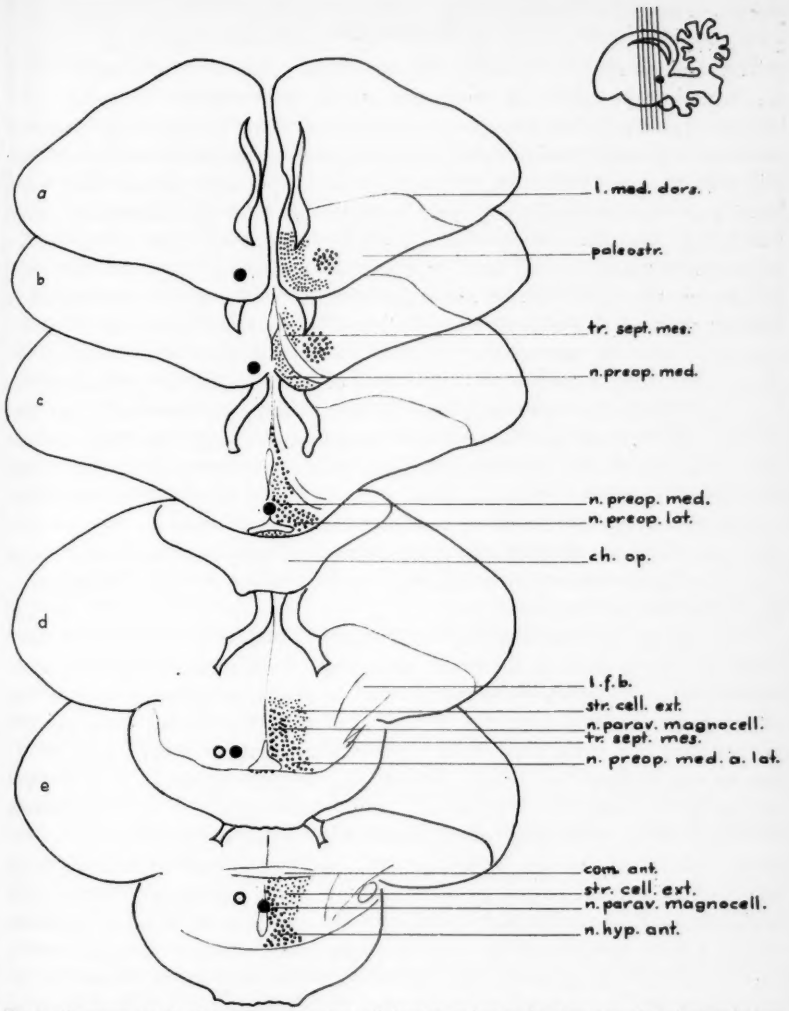

Fig. 4. Diagrams (×8) of transverse sections of the pigeon brain showing the sites of stimulation at which polydipsia was obtained as effect. The level of the sections are seen up to the right.
● = drinking during stimulation
○ = drinking after stimulation.
For abbreviations see Fig. 1.

pigeon dummies and some other postures and calls belonging to mating behaviour. Some of these responses, and particularly those related to instinctive behaviour, will be subjected to a more detailed analysis in future work.

Discussion

The results of the present study produce evidence that the central organisation of mechanisms protecting against hyperthermia and regulating food and water intake is principally the same in mammals and birds. Polypneic panting was obtained due to electrical stimulation medially in the preoptic area. In mammals this area is considered to be the site of central thermoreceptors, since local heating at this site produces polypneic panting and the mobilization of other heat loss mechanisms (MAGOUN *et al.* 1938, FOLKOW, STRÖM and UVNÄS 1949). In the pigeon panting was found to persist long after cessation of stimulation in the preoptic nuclei (Fig. 1 a and b), but at more posterior sites of stimulation panting stopped immediately on cessation of stimulation (Fig. 1 c). Panting and other heat loss mechanisms elicited by electrical stimulation in or in the close vicinity of the medial preoptic nuclei of the goat are also seen to persist for considerable time after the period of stimulation (ANDERSSON, GRANT and LARSSON 1956). Evidence has been produced that this long after-effect may be due to an after-discharge in the stimulated part of the brain stem (ANDERSSON *et al.* 1960 b). If this explanation holds true, it may be assumed that the stimulations at posterior sites, eliciting panting which stopped immediately on cessation of stimulation, affected efferent fibres from a more anterior located »heat loss centre". The latter may remain activated for different lengths of time after the end of electrical stimulation.

Hyperphagia and polydipsia were elicited by stimulations in somewhat more anterior located areas of the brain stem, than those responding to electrical stimulation with the same effects in mammals. However, as in the mammalian brain (LARSSON 1954, ANDERSSON and MCCANN 1955 b) hyperphagia was elicited from the lateral parts of the hypothalamus, whereas polydipsia occurred due to stimulations medial and anterior to the "feeding area". Stimulations on the border between the "feeding" and "drinking areas" caused pecking which was often performed into the water or directed towards the water container. The centre of the "drinking area" in the mammalian hypothalamus seems to be located lateral to the paraventricular nucleus (ANDERSSON and MCCANN 1955 b). In the pigeon drinking was seen due to stimulations in the magnocellular portion of the corresponding neurosecretory nucleus, but in this bird the "drinking area" also extended medially in front of the level of the preoptic recess. It is interesting to note that WINGSTRAND (1951) found scattered neurosecretory cells within the same parts of the avian brain. In the mammalian hypothalamus there seems to be a close anatomical and perhaps also functional correlation between the neurosecretory and the osmoregulatory systems (JEWELL and VERNEY 1957, ORTMANN 1950). The "drinking area" may be considered part of the latter system (ANDERSSON and MCCANN 1955 a and b). On the basis of the present study it may be assumed that a similar correlation between neurosecretory and osmoregulatory areas also exists in birds.

At the more lateral sites (Fig. 4 d and e) stimulation caused drinking which did not appear until stimulation was discontinued. On such occasions restlessness was seen during the actual period of stimulation. Here the stimulation may have caused an after-discharge in the "drinking area" leading to drinking as soon as disturbing side effects of the stimulation were no longer present. A similar drinking response remaining after the period of electrical stimulation is sometimes seen due to prolonged, strong stimulations of the "drinking centre" of the goat (ANDERSSON, LARSSON and PERSSON, 1960).

It has been pointed out by TINBERGEN (1951), HINDE (1953) and by several other ethologists that the instinctive behaviour of animals usually consists of two phases. When an instinct is activated, the animals show a variable initial "searching" phase of behaviour, called appetitive behaviour (CRAIG 1918). When this appetitive behaviour has brought the animal in contact with the adequate external stimuli, it is followed by a final, more or less stereotyped and relatively simple activity, called the consummatory act.

The feeding and drinking responses showed all these characteristics. When stimulated at the specific sites, the birds searched for food or water, and on finding the appropriate container, performed the final activity of feeding or drinking respectively. It could thus be shown that electrical stimulation at certain loci in the hypothalamus and preoptic area elicited integrated movements of the highest instinctive level. Recently this has also been shown in the chicken by VON HOLST and VON SAINT PAUL (1960), who elicited a wide array of instinctive actions by electrical stimulation of the brain stem.

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Analysis of Spinal Interneurons Activated by Tactile and Nociceptive Stimulation

By

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Abstract

KOLMODIN, G. M. and C. R. SKOGLUND. *Analysis of spinal interneurons activated by tactile and nociceptive stimulation.* Acta physiol. scand. 1960. *50.* 337—355. — The functional organization of spinal interneurons in the 6th and 7th lumbar segments responding exclusively to exteroceptive stimulation was studied in decapitate cats by means of intracellular technique. Seventy-six neurons were classified according to their responses to three types of skin stimuli, *viz.* touch/pressure, movement of hairs and nociceptive stimulation, into one main group responding to only one, and a second main group responding to two modalities of skin sensation. Neurons responding to all three types of skin stimulation showed also proprioceptive influence and were not included. For each modality subgroup the excitatory and inhibitory response types, receptive fields, convergence patterns, discharge patterns and histological localization of the cells are described. The dorsal region of the spinal cord showed a predominance of neurons of more simple convergence and modality type, while the medial and ventral regions contained more complex cells with contralateral and suprasegmental connections. The receptive fields were generally larger for interneurons than for afferent fibers. Convergence of various types of tactile and nociceptive inflow both from the same field and from different, often widely separated areas was demonstrated. The discharge patterns, analyzed by means of a counting rate meter, showed characteristic features for various modalities of skin sensation, excitatory as well as inhibitory responses being determined mainly by the peripheral receptor discharges. Typical phenomena of postsynaptic activity, such as spatial summation, afterdischarges and inhibitory rebound, have been exemplified and one type of selective inhibition has also been described. The various types of convergence patterns observed in neurons influenced by tactile stimulation of the paws are schematically represented in a diagram.

Spinal interneurons were classified by KOLMODIN and SKOGLUND (1954) on the basis of different convergence patterns established by adequate stimulation of proprio- and exteroceptors. Neurons with proprioceptive connections were extensively analyzed by KOLMODIN (1957). This study also included neurons showing a combined influence from muscle and skin receptors and comparisons were made between frequency-time curves of interneuron discharges during tactile and proprioceptive stimulation. Several reports on interneuron responses to sensory stimulation have appeared, *e. g.* by FRANK and FUORTES (1956), SKOGLUND and KOLMODIN (1956) and KOSTYUK (1959). In a more detailed study of background activity and evoked responses of spinal interneurons HUNT and KUNO (1959) analyzed discharge patterns both during tactile and nociceptive stimulation and also described convergence of afferent impulses from different sources. A system of cells in the dorsal horn responding to pressure and temperature changes in the skin was studied by WALL and CRONLY-DILLON (1960) who showed that the temporal pattern of discharges varied with the type of stimulus used.

The present study deals with spinal interneurons exclusively influenced by exteroceptive stimulation; the neurons studied were collected from a comparatively large material during several series of experiments (cf. HAAPANEN *et al.* 1958). Special attention has been devoted to neurons influenced by nociceptive stimulation.

Methods

As the recording and stimulation technique used has previously been described in detail (HAAPANEN *et al.* 1958; cf. also KOLMODIN 1957), only a few principal data will be recapitulated below.

The experiments have been carried out on decapitate, curarized cats maintained on artificial respiration. After laminectomy in the lumbosacral region the ipsilateral ventral roots from L_4 to S_1 were cut and mounted on electrodes for antidromic stimulation. Small amounts of Macrodex, or in some cases of blood, were injected to maintain the blood pressure. Occasional checkings of blood pressure gave values between 90 and 120 mm Hg.

The recording was made via capillary electrodes filled with 2.7—3.0 M KCl solution. An input stage of cathode follower type (HAAPANEN and OTTOSON 1954) was used in connection with a direct coupled amplifier and a double-beam oscilloscope. A counting rate meter in combination with an inkwriter (Siemens, type SD 12 K) was used for recording of the discharge frequency. Generally the time constant of the integrating circuit was kept sufficiently low (0.2 sec) to make the response time of 1 sec for the inkwriter the limiting factor. The membrane potentials were continuously recorded on a similar inkwriter; the individual action potentials were also studied and photographed on an oscilloscope screen.

At the termination of the experiment the electrode was left in its place and the spinal cord fixed *in situ* for about 12 hours in 10 per cent formalin solution. The lumbosacral part of the cord was then dissected free from the preparation and frozen sections were made. The electrode track could easily be identified under the microscope. The re-

cording sites were determined from the depths of the points along each track at which responses had been recorded. This was done for about 80 per cent of the neurons studied.

Results

Selection of material. The present investigation is based on a material of neurons that could be influenced — excited or inhibited — exclusively by cutaneous stimulation. Three different sensory modalities have been tested, *viz.* touch or pressure, hair movement and nociceptive stimuli. The sensitivity of the receptive fields to heat and cold has not been studied. We have chosen to classify the neurons in two main groups; one comprising neurons influenced by one modality of skin stimulus and a second group influenced by a combination of two modalities of skin stimuli. Influence from all three types of skin stimuli has so far only been found in cells having functional connections also with proprioceptive sources and therefore not included in this report. Within each modality subgroup the neurons have been classified on the basis of their excitatory or inhibitory responses to stimulation of various receptive fields.

The neurons described — a total of 76 — represent a selection out of a material of over 400 units and have been chosen as follows. Included were only neurons with a membrane potential not below 40 mV; the potential had to remain stable for a sufficient period to permit an analysis of convergence and discharge patterns. Excluded were all neurons showing signs of an injury, such as fast dying-out high-frequency discharges, distorted or low-amplitude action potentials and strongly fluctuating membrane potentials. Other neurons were excluded because their adequate stimulus type could not be determined with full certainty. Thus, many observations of hair and touch/pressure stimulation on hairy areas have been ruled out; as a consequence the material includes a comparatively large number of neurons activated from receptive fields located on the plantar surface, where it is easier to distinguish between these two stimulus types. As a matter of fact these areas were also of special interest in view of their significance as reflexogenic zones.

General observations. A great number of the neurons studied have been spontaneously active, *i. e.* they have not been stimulated intentionally; whether their activity has been inherent or induced by a continuous peripheral afferent impulse discharge (cf. MARUHASHI *et al.* 1952 and YAMAMOTO *et al.* 1956) is so far impossible to say. It is, however, not unlikely that a continuous interneuron activity is set up by receptors close to operative wounds and fixation points or from thermoreceptors, or from proprioceptors that may have been overlooked in tests using muscle stretch (cf. KOLMODIN 1957). No attempts have been made to analyze this spontaneous activity, but some observations made may be worth mentioning. (*i*). An initially silent neuron, which could be brought to discharge only in response to a peripheral stimulus, could after some time of activation maintain a discharge in the absence of any stimulation or

any visible damage within the skin area stimulated. This sustained activity could then be increased by renewed application of the adequate peripheral stimulus. (*ii*). The number of initially active neurons is considerably reduced by a lowering of the blood pressure to about 80 mm Hg or by even a very insignificant damage of the spinal cord vessels causing changes in the local blood supply (cf. KOLMODIN 1957). On the other hand, the discharge frequency of spontaneously firing neurons seems to be only little influenced by moderate variations in blood pressure.

In an analysis of results from experiments using single unit recording it must be taken into consideration that the excitability of each neuron in the central nervous system is incessantly changing. These variations arise, in part, from humoral factors playing upon the neurons but they are also very likely caused by changes in the impulse milieu of the neuron due to the varying number of impulses impinging upon it via the connections open at the time. Thus, for instance, the slow fluctuations in the membrane potential of the neurons often seen in lengthy intracellular recordings are no doubt due to variations of this kind (KOLMODIN and SKOGLUND 1958). It is not uncommon that a neuron, which initially is being excited or inhibited by a particular afferent source, ceases to respond to this source after some minutes of recording and then, after another few minutes, reacts to it once more. Strong stimuli, *e. g.* electric shocks to peripheral nerves, have also been observed to result in transient or more long-lasting changes in the discharge pattern of a neuron or in its pattern of afferent connections.

The investigation has not aimed at determining the anatomical or functional system to which the neurons belong. Owing to the axon collaterals described for many types of spinal interneurons (CAJAL 1909) one cell may serve both ascending tracts and segmental reflexes. On the basis of the histological determinations of the recording sites the neurons have been localized to five main areas of the spinal cord cross section, as indicated in Fig. 1, to which references will be made in the following. As a general finding, cells in the dorsal region I showed less complex connections than those of regions II—IV.

Group I.

Neurons influenced by one modality of skin sensation

The fifty neurons in this group were divided in subgroups as follows:

A. Neurons influenced by touch/pressure

Adequate stimulation for the twenty-three neurons classified in this group was touch or light pressure applied by the finger or by a small glass rod. In most cases light pressure was applied, since attempts proved that a distinction between touch and light pressure was not only very time-consuming but also

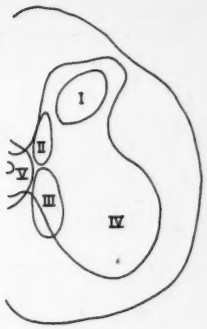

Fig. 1. Schematic cross section of lumbar spinal cord indicating the regions (I—V) in which the various interneurons studied were located. See text.

as a rule impossible in practice. As mentioned above, touch/pressure stimulation has chiefly been applied on hairless areas, in the first line the pads.

Neurons excited. In nineteen of the neurons influenced by touch/pressure an excitatory effect was obtained on stimulation, *viz.* either a discharge in an initially silent cell or an increase of a spontaneous activity. The receptive fields of these neurons varied from one small pad or one big pad to all the small pads and, in some cases, all the pads of the ipsilateral hindpaw. Some units were also found with several, widely separated receptive fields, in most cases located in the pad region of two to three different paws. Incidentally some neurons were observed that could be influenced from an area in the perineal region. Units activated from the ipsilateral pads were mostly found in region I (cf. Fig. 1) but may also occur in regions II—IV, those influenced from the contralateral hindleg in the ventromedian region III, while neurons activated both from the contra- and ipsilateral forelegs were located in regions II and III. The commissural region V contained cells activated from receptive fields in the perineal region.

The postsynaptic response to touch was a short train of impulses lasting up to 2 or 3 sec, after which the unit ceased to discharge or resumed its spontaneous activity. The frequency of these discharges varied between 5 and well over 100 impulses per sec. As a rule, the highest frequencies in response to touch stimulation were recorded from units with a spontaneous activity of comparatively high frequency.

The most common response to application of steady pressure was a rapid rise in frequency to a rather high rate. Within some seconds the discharge frequency declined to a more or less steady state, and then either continued at this rate or very slowly declined during the stimulation period, Fig. 2 *A*. The curve in *B* shows the same rapid initial rise in frequency as in *A* but in this case the discharge declines to a very low frequency which is not maintained for more than about 10 sec and then dies out in spite of continued ap-

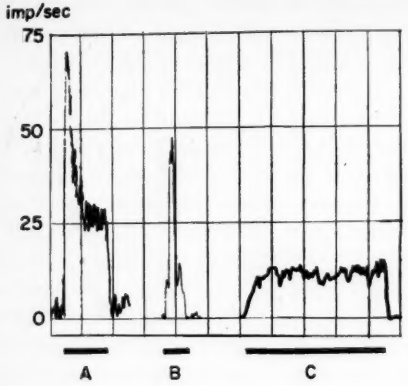

Fig. 2. Frequency-time curves from three different interneurons (*A*, *B* and *C*) illustrating various types of responses to steady pressure applied to pad of ipsilateral hindpaw. Time between vertical lines 30 sec. Lines below records in this and following figures indicate approximate stimulus duration.

plication of the stimulus. Record *C* illustrates a more unusual case, with no initial transient rise in frequency.

A few units were observed which responded to steady pressure of the pad with a short burst of impulses but which could not be activated by light touch.

Neurons inhibited. In four neurons application of pressure led to inhibition of a spontaneous activity. In these cases no area could be found from which the unit could be excited. The receptive fields consisted of an ipsilateral pad or an area in the perineal region. One of the neurons was located in region III in Fig. 1; the site of the others was not identified. Fig. 3 illustrates the inhibitory response of one of these neurons which showed a slowly fluctuating spontaneous discharge of about 30 impulses per sec. Steady pressure applied to the big pad of the ipsilateral hindpaw (*A*) resulted in a slowing down of the spontaneous discharge frequency to about 15 per sec. The inhibitory stimulation was maintained for more than 90 sec but in spite of the constant pressure the discharge frequency increased slowly up to a more or less steady level of about 25 impulses per sec. A neuron may thus adapt to an inhibitory sustained stimulation in the same way as to an excitatory stimulation.

The curve also illustrates another phenomenon observed in several neurons with inhibitory properties, *viz.* post-inhibitory rebound. As seen in the figure, release of the stimulus caused a transient increase of the discharge frequency to about 40 per sec before the spontaneous frequency of about 30 per sec set in again.

The latter part of the curve in Fig. 3 illustrates an observation made in the analysis of this neuron — as in some other cases — with respect to the effect of electrical stimulation. In the experiment a short part of the intact sural nerve had been dissected free and hooked up on stimulating electrodes. Stimulation of the nerve by electric single shocks at intervals of several seconds had no significant effect on the spontaneous discharge. Nor had a repetitive

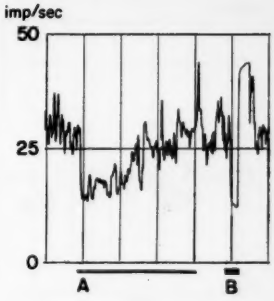

Fig. 3. Frequency-time curve from spontaneously active interneuron illustrating inhibitory effects of: *A*, steady pressure to big pad of ipsilateral hindpaw; *B*, repetitive electrical stimulation of ipsilateral sural nerve at 50 per sec. Time 30 sec.

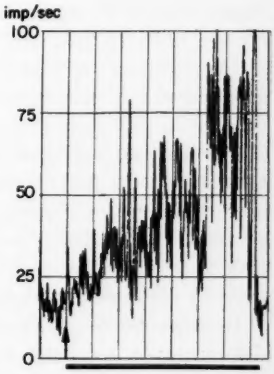

Fig. 4. Interneuron discharges of successively increasing frequency caused by repeated stroking of hairs on calf of ipsilateral hindleg. Time 30 sec.

electrical stimulation of the nerve any effect as long as the stimulus frequency did not exceed 30 per sec, but above this frequency there was an immediate decline of the spontaneous discharge to about 12 impulses per sec. On release of the electrical stimulation there was again a post-inhibitory rebound; the discharge frequency rose to more than 40 per sec before resuming its spontaneous rate of about 30 per sec.

B. Neurons influenced by movement of hairs

Adequate stimulation for the nine neurons classified in this group was movement of the hairs on a skin area; an *excitatory* effect resulted in all cases. The receptive fields of these neurons varied in size; in some cases they were comparatively small (about 1 cm^2) and located on the peripheral parts of the limbs, *e. g.* between the pads or on areas on the plantar side of the paw; in other cases the receptive fields were very large, comprising *e. g.* the sole of the foot or an area covering the posterior part of the leg and thigh ipsilaterally, or the whole tail. Neurons with small receptive fields were found in the areas

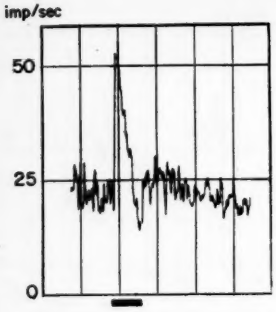

Fig. 5. Interneuron response to nociceptive stimulation (pinching of pad of ipsilateral hindpaw). Time 30 sec.

marked I and II in the schematic drawing Fig. 1, and those with large receptive fields in area III.

The neurons were either spontaneously active or initially silent and responded to hair movement with a short train of spikes. A steady stimulation did not in any case give rise to a sustained discharge of the type described above for touch/pressure stimuli. However, by quickly repeated strokes or blowings on the hairs a discharge of comparatively high frequency and with large frequency variations could be built up in some cases. An example is given in the frequency curve in Fig. 4. Initially, the spontaneous discharge frequency varied between 15 and 20 per sec but it could be increased to well over 75 per sec by repeated strokes of the hairs.

In some neurons with large receptive fields spatial summation could be demonstrated, *viz.* stroking of the hairs of the whole receptive field resulted in an impulse frequency twice as high as if only part of the area was being stroked. Some of the neurons with large receptive fields could be driven optimally from a certain part of the area only; thus, one unit with a receptive field comprising the whole tail could be driven to 60 impulses per sec from an area on the distal part of the tail, whereas stimulation of more proximal parts resulted in only 40 impulses per sec.

C. Neurons influenced by nociceptive stimulation

In this group have been classified eighteen neurons influenced by pinching or pinpricks on a skin area but not responding to stimuli such as hair movement, touch or steady pressure. The difficulties encountered in trying to distinguish between nociceptive stimulation and other stimuli are obvious, but it has been considered reasonably safe to characterize the stimulus types used in these experiments as nociceptive because, when applied to the intact or decerebrate cat, they evoke warding-off and reflex movements.

Neurons excited. The fourteen neurons in which excitatory effects were elicited could be roughly divided into two types, after the location of their receptive fields. The units of one group had receptive fields comprising one compara-

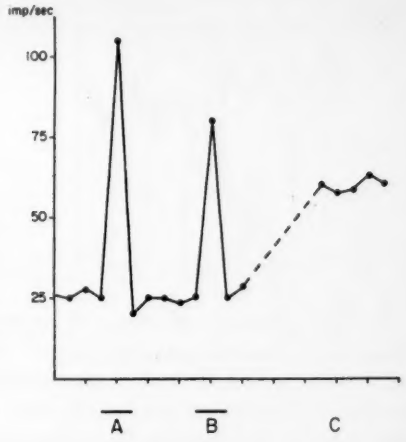

Fig. 6. Maximum responses of different frequencies to nociceptive stimulation of two different receptive fields. *A*, pinching of area round base of tail; *B*, pinching of pad of ipsilateral hindpaw; *C*, increase of spontaneous discharge frequency after end of stimulation. Graph based on values from original frequency-time curve. Time 30 sec.

tively small area, *e. g.* one big pad or two small pads with an adjacent hairy skin area of a size of one or a few cm^2, or the outermost part of the tip of the tail; these areas were as a rule located on the ipsilateral hindleg but have also in some cases been observed on the contralateral hindleg. The units of the other group could be driven from widely separated receptive fields, usually two, one of which located, *e. g.*, on the ipsilateral hindpaw and the other on some part of the tail; each such area had about the same size as those in the first group.

The neurons excited by nociceptive stimulation were, with two exceptions, spontaneously active. All units tested by means of pinprick stimulation responded by a transient increase of the discharge frequency lasting less than 1 sec. Application of a sustained nociceptive stimulation, *e. g.* repeated pinching of a skin area, resulted in some cases in similar discharges as pinprick stimulation; in other cases the result was a marked increase in the discharge frequency which only gradually declined to the initial level. An example is given in Fig. 5. As appears from the figure, this neuron was spontaneously active with a discharge frequency of about 20 to 25 per sec. Pinching of the pad of the ipsilateral hindpaw resulted in a rapid increase of the discharge frequency up to 55 per sec, followed by a stepwise decline in the course of about 20 sec to the pre-existent frequency. When the stimulus was released a further transient decline of the response frequency resulted, after which the neuron resumed its spontaneous frequency.

Fig. 6 shows a frequency-time curve obtained from a neuron influenced from two separate receptive fields. For neurons of this type the maximum response on stimulation of one of the receptive fields often exceeded that obtained from the other receptive field. The unit recorded in Fig. 6 had a

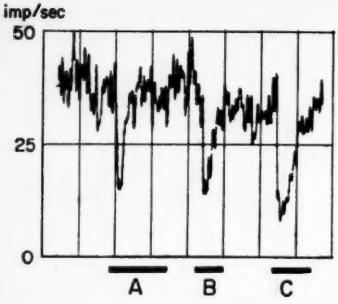

Fig. 7. Inhibitory effects of nociceptive stimulation from different receptive fields. *A*, pinching of small pad of ipsilateral hindpaw; *B*, pinching of pad of contralateral hindpaw; *C*, pinching of area on distal part of tail. Time 30 sec.

spontaneous activity of about 25 impulses per sec. The maximum response frequency elicitable from the receptive field on the tail was 105 per sec (*A*), while the response frequency after pinching of the pad could not be brought to exceed 80 impulses per sec (*B*). The spontaneous activity toward the end of the curve rose to about 60 impulses per sec (*C*), a phenomenon which may be analogous to that described above (p. 339) of an initially silent neuron starting to discharge continuously after some time of skin manipulations. However, in this case it cannot be excluded that the nociceptive stimulation has damaged the peripheral tissues, thus causing an increase of the afferent inflow.

Neurons inhibited. The receptive fields in which nociceptive stimulation evoked an inhibitory effect were either located at one limited skin area on the tail or on the ipsilateral hindleg (size 1—5 cm^2) or at various sites, as the pads of different paws or the tail. The four neurons in this group were spontaneously active, the impulse intervals being usually very irregular. A brief nociceptive stimulation gave rise to a transient decline of the discharge frequency but only rarely to complete inhibition. The most common type of response to a steady nociceptive stimulation resembled that elicited in neurons inhibited by steady pressure, *viz.* the response adapted slowly to the stimulation. An example is given in Fig. 7. This neuron was discharging spontaneously at a rate of 30—40 per sec; application of constant pinching to a small pad of the ipsilateral hindpaw reduced the initial frequency momentarily to 15 per sec, after which there was a slow increase in the course of 20 sec back to the initial level. On release of the inhibitory stimulus the frequency slightly increased, a phenomenon of the same type as that observed after pressure stimulation (cf. Fig. 3). The curve in Fig. 7 also gives examples of the discharge pattern when the inhibitory effect is elicited from two other receptive fields. In principle, the effects are the same in all cases.

All neurons excited by nociceptive stimulation of one receptive field were located in the regions marked I, II and III in the schematic drawing Fig. 1, while those excited from two separate receptive fields were only found in region III. Neurons inhibited by nociceptive stimulation were localized to the ventral horn (IV), at the boundary between white and grey matter.

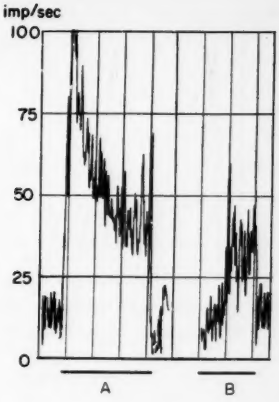

Fig. 8. Interneuron responses to different modalities of skin stimuli. *A*, slowly adapting discharge in response to light constant pressure (200 g) to big pad of ipsilateral hindpaw; *B*, "built-up" discharge in response to repeated manipulations of hairs between pads of same paw. Time 30 sec.

Group II.

Neurons influenced by two modalities of skin sensation

The material comprises twenty-six neurons influenced by two of the three types of stimulation used, which were divided in subgroups as follows.

A. Neurons influenced by hair movement and touch/pressure

Neurons excited. The receptive fields of the sixteen neurons of this group were in all cases except one located on the ipsilateral hindpaw and consisted of (i) one or more pads, with touch/pressure as adequate sensory stimulus, and (ii) the hairy area between these pads, sometimes extending to the plantar side of the foot, and with hair movement as adequate sensory stimulus. These neurons were thus influenced from receptive areas on one paw. One neuron had three separate receptive fields, located on the forepaws and the paw of the contralateral hindleg, each field comprising the pad and a hairy area on the plantar side of the paw. In all these neurons only excitatory effects could be evoked. Those influenced from one receptive field were localized to the region marked II in Fig. 1, and the unit influenced from three separate fields was found in area III, at the boundary between white and grey matter.

Fig. 8 shows an example of the discharge patterns of these neurons. *A* is the response to a light constant pressure (200 g) applied to the big pad of the ipsilateral hindpaw. The discharge is very irregular and variable, but it is clearly seen how the response frequency after the initial rapid increase is slowly adapting to the constant stimulation and after about 60 sec reaches a fairly steady level. The discharge pattern is of the same type as in the units described above responding only to pressure. Hair movement elicited in this unit a short burst of fast adapting activity, but by means of rapid repeated stroking of the hairs a discharge could be built up to a frequency varying

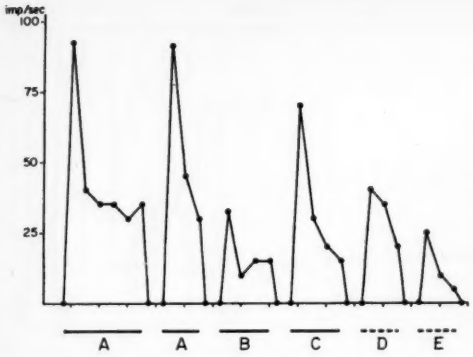

Fig. 9. Maximum responses of different frequencies to pressure stimulation (*A*—*C*), and to hair stimulation (*D*—*E*), applied to different receptive fields. *A*, pressure of big pad of contralateral hindleg; *B*, same on contralateral foreleg; *C*, same on ipsilateral foreleg; *D* and *E* movement of hairs between pads of contralateral hind- and forelegs respectively. Graph based on values from original frequency-time curve. Time 30 sec.

between 25 and 45 impulses per sec (*B*). The discharge pattern is similar to that in Fig. 4. As might be expected, the discharge type thus depends to a high degree on the modality of the stimulus.

Fig. 9 shows an example from a neuron with widely separated receptive fields. *A* is the response to pressure applied to the big pad of the contralateral hindleg, *B* the response to the same stimulation applied to the big pad of the contralateral foreleg, and *C* to the ipsilateral foreleg, while *D* and *E* are responses to hair movement between the pads of the contralateral hind- and forelegs respectively. In all these cases the stimulus was adjusted so as to give a maximum response. The maximum frequency obtained by repeated tests on one and the same area was rather constant (*A*), whereas tests on different areas may result in a frequency three times as high in one receptive field as in another, even for stimuli of the same modality.

B. Neurons influenced by touch/pressure and nociceptive stimulation

Ten neurons were found that could be influenced both by touch/pressure and by nociceptive stimulation. In six of them only excitatory effects were elicitable, in the other four both excitatory and inhibitory effects.

Neurons excited. For the neurons with purely excitatory properties the receptive fields for touch/pressure were found on the three lateral small pads of the ipsilateral hindpaw, while areas sensitive to nociceptive stimulation were found on the corresponding pads of the contralateral hindpaw and on the distal two-thirds of the tail; adequate stimulation of any of these areas led to an increase of the discharge frequency.

In one of these neurons a summated response could be obtained by simultaneous stimulation of a pain-sensitive area and another touch-sensitive area, as is illustrated in Fig. 10. *A* is the maximal response obtained by pressure of a small pad, and *B* the maximal response to hard pinching of the tip of the

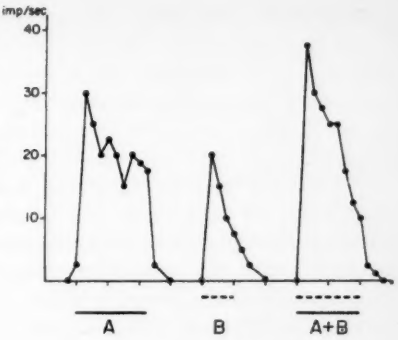

Fig. 10. Summation of interneuron responses to tactile and nociceptive stimulation of different receptive fields. *A*, steady pressure of small pad of ipsilateral hindpaw; *B*, hard pinching of tip of tail by means of forceps (this region did not respond to pressure or touch only); *A* + *B*, simultaneous application of the two stimuli. Time 30 sec.

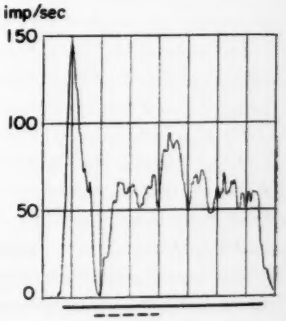

Fig. 11. Inhibitory effect of tactile stimulation on activity set up by nociceptive stimulation. Solid line: constant hard pinching of area on tail. Dotted line: steady pressure applied to pad of ipsilateral hindpaw. Time 30 sec.

tail. It appears that simultaneous application of the two stimuli results in a discharge of significantly higher frequency.

Neurons excited/inhibited. Two neurons could be excited by nociceptive stimulation of one area and inhibited by application of touch/pressure to another area. One of these neurons, illustrated in Fig. 11, was initially silent. Nociceptive stimulation by hard pinching of the tail elicited a postsynaptic discharge at an initial frequency of about 150 impulses per sec. The neuron adapted stepwise to the stimulation and after about 30 sec the discharge frequency had fallen to 60 impulses per sec. Constant pressure was then applied to the ipsilateral pad, resulting in an immediate, complete inhibition of the activity; after a second or two, however, the activity set in again, gradually reaching a comparatively steady level at a frequency of about 60 impulses per sec. On release of the inhibitory stimulation the neuron responded with a transient increase of the discharge frequency. After termination of the nociceptive stimulation there was an afterdischarge for about 10 sec and then the neuron was silent again.

In two neurons touch/pressure set up an activity which could then be inhibited by nociceptive stimulation. One of these neurons merits a description

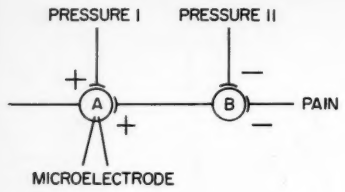

Fig. 12. Possible coupling of interneurons to explain selective inhibition. Plus sign = excitatory effect; minus sign = inhibitory effect. See text.

as having a comparatively complex integrative pattern. This neuron was spontaneously active and its discharge frequency could be increased by pressure on the big pad of the ipsilateral hindpaw. The spontaneous activity could be completely inhibited by a moderate pressure on a small pad of the same paw and also by nociceptive stimulation of an area of the tail. However, none of these inhibitory stimuli had any effect on the activity set up by stimulation of the excitatory source (the big pad). To explain the selective inhibition thus observed, various coupling patterns may be suggested, one of which is represented in Fig. 12. *A* is the neuron recorded from, which is directly excited by pressure (I) on the big pad. Its spontaneous activity is assumed to be maintained by activity from another neuron (*B*). It is obvious that if impulses from the two inhibitory stimuli, pressure (II) and pain, converge on *B* a selective inhibition of the spontaneous activity in *A* results from activation of these sources. Analogous explanations of a selective inhibition of this type can be made on the basis of presynaptic interaction (cf. HOWLAND *et al.* 1955) or on the assumption of different sites of excitation and inhibition in the neuron recorded from, *e. g.* cell body and dendrites (cf. SPRAGUE 1958).

Neurons influenced by hair movement and nociceptive stimulation have been observed in a few instances, but the material does not allow of a detailed description.

Discussion

In earlier investigations on spinal interneurons it has generally been found that both the spontaneous and induced activity is of a characteristically irregular rhythm (KOLMODIN and SKOGLUND 1954, FRANK and FUORTES 1956, KOLMODIN 1957, HUNT and KUNO 1959). HUNT and KUNO found, however, by analysis of the frequency distribution of impulse intervals that many spontaneously discharging units showed frequency variations which were not purely random but indicated a rhythmic generation of impulses. In the present experiments no such rhythmicity was recorded which may be due to the time constants of the counting rate meter and recording devices. In studies on decerebrate cats FRANK and FUORTES (1956) observed regular cycles of frequency modulation recurring at rates of 0.5—10 per sec. Frequency variations of this type have not been observed in the present study on decapitate animals, which

suggests that, normally, centers in the brainstem may exert a modulating effect on spinal interneuron activity.

All neurons influenced by movement of hairs responded to a sustained stimulation by a short burst of impulses; a fast adapting response of this kind was actually to be expected, the receptors involved being also rapidly adapting (ZOTTERMAN 1939). Neurons responding to sustained pressure showed a sudden initial increase of the discharge frequency and then adapted to a fairly constant lower frequency level. The frequency curve of these neurons was thus, in principle, similar to that of the receptors activated (cf. ADRIAN and UMRATH 1929). Some of the neurons responding to nociceptive stimuli showed a rapidly adapting response to sustained stimulation, while in others the pre-excitatory frequency level was attained only after 10—15 sec. This is in accordance with the findings of both rapidly and slowly adapting receptors in the cat's hindpaw (ZOTTERMAN 1939, MARUHASHI *et al.* 1952). In a study of interneurons with proprioceptive connections (KOLMODIN 1957) a statistical comparison was made between the average frequency-time curves of discharges in afferent neurons and interneurons during application of a standardized stretch stimulation of a muscle. It was then found that when the average afferent curve had reached a constant level there was still a continued frequency fall in the interneuron curve, and this phenomenon was interpreted as being due to "central adaptation". Unfortunately the more varying and less fiber-specific afferent inflow from skin receptors makes it difficult to perform such an exact comparison for interneurons with exteroceptive connections.

In investigations on the cat's sensory units MARUHASHI *et al.* (1952) found that the receptive fields for touch and pressure fibers especially on hairless areas were small (below 1—2 mm^2), while those for fibers associated with hairs were larger (1—2 cm^2). The receptive fields of nociceptive fibers were found to vary between 2 and 50 mm^2, the small fields being located on the toe pad and the plantar cushion, the larger ones on hairy skin areas. On the whole, YAMAMOTO *et al.* (1956) have reported similar results and found, besides, that the most distal receptive fields for tactile stimulation were smallest and that the fields became larger according as their position was more proximal. As a rule, the interneurons studied in this investigation were found to have larger receptive fields than the primary afferent neurons, in some cases with widely separated areas. Thus, *e. g.*, the receptive fields for touch/pressure stimulation are considerably larger, comprising one or more pads, sometimes on different paws, which implies that there is a marked convergence even on neurons influenced by one modality of skin sensation. The receptive fields for tactile stimulation seem to become smaller the more distal they are, even though this does not apply to the central neurons in the same general way as for peripheral fibers. Also the receptive areas for nociceptive stimulation have as a rule been larger than corresponding fields of primary afferent neurons. This seems to be the case especially for receptive fields located on distal parts of

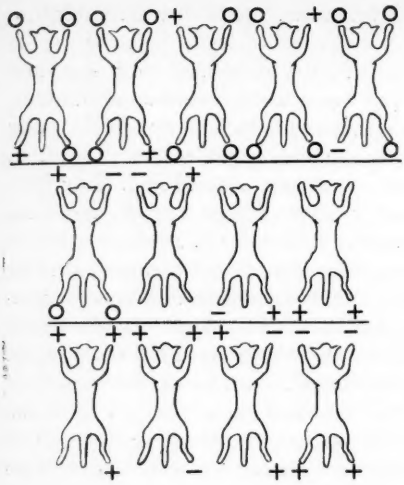

Fig. 13. Schematic diagram of convergence patterns of neurons influenced by tactile stimulation of the plantar surface of paws. Each schematic figure represents a convergence type observed in at least two neurons. Neuron recorded from always on left side of lumbar cord. Plus sign = excitation; minus sign = inhibition; zero sign = no influence and absence of sign = no test.

the body. In most cases the sizes of the receptive fields for tactile stimulation agree fairly well with those found for neurons in thalamus and the somatic sensory cortex (GAZE and GORDON 1954, MOUNTCASTLE 1957). In this investigation we have only exceptionally found neurons with receptive fields of the large size reported for neurons in the ventral spinocerebellar tract (OSCARSSON 1957).

Recent investigations of the convergence patterns of individual nerve cells at higher levels of the sensory system have revealed a rather varying organization. Thus, *e. g.*, neurons in certain parts of thalamus (GAZE and GORDON 1954) and in the sensory cortex of cat (MOUNTCASTLE 1957) seem to be modality-specific and to be driven from comparatively limited receptive fields, whereas neurons in certain cortical somatosensory association areas may be activated from several spatially separate afferent sources (AMASSIAN 1953). In other centers, as *e. g.* certain parts of the reticular formation (BAUMGARTEN and MOLLICA 1954, SCHEIBEL *et al.* 1955), there is an extensive convergence on single neurons of different modalities. The main principles for convergence of proprio- and exteroceptive pathways on spinal interneurons have earlier been established (KOLMODIN and SKOGLUND 1954, KOLMODIN 1957), and the present investigation has shown the varying types of convergence of exteroceptive inflow that may occur in the spinal cord. It seems possible that the convergence patterns observed for neurons in higher centers are in part formed even at the spinal level.

Provided the interneurons studied are engaged in ascending sensory systems the typical findings of a convergence of tactile and nociceptive inflow may

be relevant to certain problems of sensory integration. It has been suggested that the hyperpathia occurring in patients with certain peripheral nerve lesions may be attributed to a specific reduction in the afferent inflow which in turn would cause the disturbance in the processes of sensory integration and perception (WEDDELL *et al.* 1948). This assumption may get experimental support from the present investigation, from which it is obvious that an interneuron may exhibit a quite different discharge pattern if one modality of afferent inflow is reduced. The consequences of such a functional disturbance are likely to be more serious if an inhibitory influence is eliminated.

The pathophysiological basis of referred pain is little known, but one current theory presumes a convergence of sensory inflow from different sensory areas on one and the same central neuron. The actual finding of convergence of pain impulses from widely separated exteroceptive areas on single interneurons even at the spinal cord level is of interest in this connection.

The interneurons studied may also serve as links in spinal reflex systems, and from this concept the functional significance of some special types of convergence patterns will be considered. Fig. 13 gives a survey of the various convergence patterns found in interneurons in the lumbar cord influenced by tactile stimulation of the plantar side of the paws. The upper row of pictures shows various types of simple functional connections with sensory areas on one paw only, while the following rows show convergence patterns of increasing complexity. It appears that only part of the possible variations in excitation-inhibition patterns have been found so far, but it seems likely that analysis of a larger cell material will reveal that all types are represented in the interneuron pool of the lumbar cord. The convergence from sensory areas of the forepaws on neurons in this region (KOLMODIN and SKOGLUND 1954) is of special interest and suggests that the neurons are links in the long spinal reflex system connecting fore- and hindlegs with one another (SHERRINGTON and LASLETT 1903). In his analysis of these reflexes LLOYD (1942) found that impulses induced in a foreleg reached the lumbar segments via uncrossed, decussated and double decussated paths. The interneurons influenced from the forelegs were located in the ventral part of the cord, chiefly in the medial region, in nucleus cornu-commissuralis anterior. The present data accord well with these findings. The schematic drawing of the convergence patterns gives a picture of how a reflex system engaged in the coordination of movements of the different extremities may be built up. It should be kept in mind that the present results have been obtained on decapitate animals, and it is probable that in the intact animal the functional connections of the neurons are still more complex.

Among cells with combined exteroceptive and proprioceptive connections, not included in this description, there are some of a relatively simple type with only one or two exteroceptive sources in combination with proprioceptive sources, as has been exemplified in the work of KOLMODIN (1957). However,

our unpublished material also includes examples of cells of a more complex organization, with inflow from several skin areas. These neurons can sometimes be activated by all three modality types of skin sensation tested in this work, which is of interest since in the present material only a combination of two modalities has been observed; it is possible that this may be due to the limited material tested. A distinction between cells with and without proprioceptive connections is of course rather artificial from a functional point of view and, besides, it is difficult to draw safe conclusions in this respect under the experimental conditions employed. That neurons with purely exteroceptive connections deserve a special study may, however, be evident from the account given above.

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From the Institute of Physiology, University of Lund, Sweden

Functional Organization of the Dorsal Spino-Cerebellar Tract in the Cat

VII. Identification of Units by Antidromic Activation from the Cerebellar Cortex with Recognition of Five Functional Subdivisions

By

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Received 24 July 1960

Abstract

Lundberg, A. and O. Oscarsson. *Functional organization of the dorsal spino-cerebellar tract in the cat. VII. Identification of units by antidromic activation from the cerebellar cortex with recognition of five functional subdivisions.* Acta physiol. scand. 1960. *50.* 356—374. — Fibres of the dorsal spino-cerebellar tract have been identified by antidromic activation from the anterior cerebellar lobe cortex and classified in 5 subdivisions activated: 1) from muscle spindles, 2) from Golgi tendon organs, 3) from pressure receptors in the pad, 4) on touch, pressure and pinching of the skin, 5) as 4) and in addition from high threshold muscle afferents.

In the first papers of this series ascending pathways in the dorsal part of the lateral funiculus (Flechsig's fasciculus) were analyzed by recording the mass discharge in the dissected fasciculus and by unit recording from single axons. The neurones were classified with respect to the actions evoked by primary afferents, investigated both with electrical stimulation of nerve trunks and with adequate stimulation of receptors (Laporte, Lundberg and Oscarsson 1956 a, b, Laporte and Lundberg 1956). In further experiments special attention was given to neurones which could be assumed to belong to the dorsal spino-cerebellar tract (DSCT) because an evoked potential in the cerebellar cortex corresponded to the discharge in these axons. These were the

neurones monosynaptically activated by group I muscle afferents. Two subgroups were found, on one hand those activated by muscle spindle afferents (Ia + II) and on the other those activated by the Ib afferents from Golgi organs (LUNDBERG and OSCARSSON 1956, HOLMQVIST, LUNDBERG and OSCARSSON 1956, LUNDBERG and WINSBURY 1960). Intracellular recording from Clarke's cells activated by these afferents was made by CURTIS, ECCLES and LUNDBERG (1958).

These investigations have been extended. Antidromic activation from the cerebellar cortex has been employed and three new functional subgroups have been recognized. Three pathways with axons ascending in the dorsal part of the lateral funicle, but not belonging to the DSCT, will be described separately (LUNDBERG and OSCARSSON 1960). A preliminary account of some of the findings has been given (LUNDBERG and OSCARSSON 1959).

Methods

The experiments were made on unanaesthetized cats decerebrated by intercollicular section. Part of the anterior cerebellum was exposed for stimulation and laminectomies were made at three levels. 1) In L4—L5 for recording of the incoming volley from the dorsal column and stimulation of axons in the lateral funicle to identify neurones with cells below the caudal level of Clarke's column. 2) In L1 for microelectrode recording. 3) In Th8 for antidromic stimulation of axons and also in a number of experiments for recording the mass discharge in the dissected contralateral spinal half. In all experiments the dorsal column was removed at this upper level for a distance of about one cm. For the technique of recording see LAPORTE *et al.* (1956 a, b).

In most experiments the cutaneous nerves were dissected for stimulation but left in intact connection to permit adequate stimulation of skin receptors; only two skin nerves were regularly sectioned, the lateral nerve (traversing the lower part of biceps femoris) and the small cutaneous branch of the deep peroneal nerve also containing fibres to extensor digitorum brevis. The saphenous nerve was not dissected for stimulation. In most of the experiments the tibial nerve (peripheral to the nerve to flexor digitorum longus) was prepared for stimulation but not sectioned to permit investigation of adequate effects from the foot. The animals were kept under flaxedil and artificially respirated.

Square wave pulses of a duration of about 0.2 msec were used for stimulation of the cerebellar cortex of which only culmen was exposed. A single movable wick electrode (cathode) was used and the indifferent electrode was placed in the temporal muscle. For each unit the cerebellar cortex was explored during continuous repetitive stimulation (100/sec) at a rather high stimulus strength to ascertain if the fibre could be antidromically activated. If so the area from which the effect could be evoked at a slightly supraliminal strength was demarcated and the threshold measured. Many fibres were activated at 0.5 volt or less from an area of perhaps one square mm, which was surrounded by cortex from which no antidromic activation could be obtained at this strength. However, with a number of units antidromic activation was only possible from the anterior margin of the exposed anterior cerebellum. In those cases the threshold was often higher but was accepted as evidence for cortical termination if activation occurred at 2 volt or less and after ascertaining that the fibre could not be stimulated from the inferior colliculi.

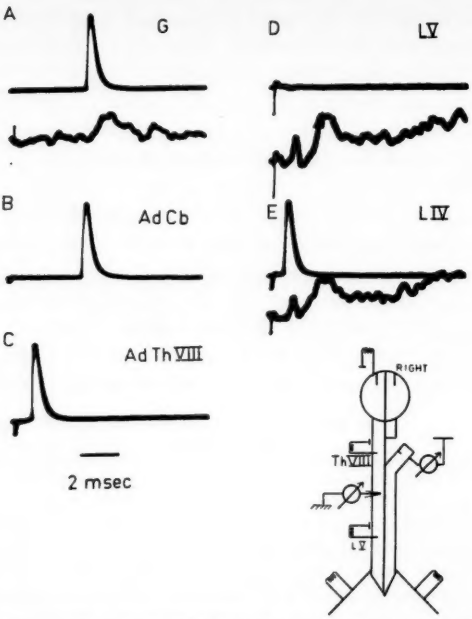

Fig. 1. Microelectrode recording from DSCT axon (upper trace) and recording from the contralateral spinal half (lower trace in A, D and E). Record A shows the effect of a group I volley from the nerve to the left gastrocnemius-soleus (G). In record B the intermediate cortex of the anterior lobe was stimulated. The left lateral funicle was stimulated at the indicated levels in records C—E. Distance from the site of microelectrode recording to: stimulating electrode in L5 5.1 cm, stimulating electrode in L4 4.0 cm, stimulating electrode in Th8 5.7 cm, anterior cerebellum 22.1 cm.

Results

1. *Procedure of identification*

Fig. 1 illustrates the procedure used in these experiments. The unit was monosynaptically activated by a group I volley from the nerve to gastrocnemius-soleus (record A). The axon could be antidromically activated on stimulation of the lateral funicle in Th8 (record C) and from the intermediate cortex of the anterior cerebellum (B) but not by a strong shock applied to the lateral funicle in the upper part of the fifth lumbar segment (D). The stimulus in L5 was strong enough to excite fibres in the dorsal column resulting in synaptic activation of neurones with axons ascending in the contralateral spinal half (lower trace in D). In this investigation none of more than 200 DSCT axons, identified by antidromic stimulation from the cerebellar cortex, could be excited by a stimulus applied to the lateral funicle in the rostral part of the fifth lumbar segment. This is in agreement with the fact that Clarke's column in cat never extends beyond the 4th lumbar segment (Rexed 1954).

When in the experiment of Fig. 1 the stimulating electrode was moved 11 mm proximally to the upper part of L4 the axon could be activated (E). Accordingly this axon originated from a cell in the most caudal part of Clarke's column. This was only found with few DSCT axons and would not be expected often since Clarke's column tapers quickly in the 4th lumbar segment or actually may end at the transition between L3 and L4 (REXED 1954). Furthermore it should be remembered that sacral and lower lumbar afferents supply Clarke's column cells of a number of segments (SZENTÁGOTHAI and ALBERT 1955, LIU 1956).

The latencies of the spike in C and E indicate a conduction velocity of 80 m/sec. With a uniform conduction velocity in the axon this would correspond to a latency of 2.8 msec on cerebellar stimulation, whereas the actual latency in B is 3.5 msec. This was a regular finding with the axons of all DSCT subgroups described in this paper and the time difference was usually of the above order. In part this may be due to slow conduction in terminal branches but presumably not entirely because the latency did not shorten more than about 0.2 msec when the stimulus applied to the cerebellar cortex was strongly increased. Tapering of the fibres possibly in connection with collaterals given off subcortically may be the explanation.

The DSCT fibre of A—F, Fig. 2, had a conduction velocity of 42 m/sec as calculated from record E and correspondingly the latency from cerebellum in F is longer, but actually only by 0.5 msec exceeding the latency calculated from the velocity of 42 m/sec. With some slow fibres a larger discrepancy was found. The slow conduction velocity in the axon of Fig. 2 was not at all an exceptional finding. Our findings show that the calibre spectrum of the DSCT is rather wide. Conduction velocities were distributed from about 100 m/sec down to 30 m/sec. The latter value would correspond to a diameter of 5.0 μ (provided that the conversion factor 6 of HURSH (1939) is valid for fibres in the CNS). There was no difference between the different subgroups in this respect.

2. *Units activated by group I muscle afferents*

Out of 100 axons activated by group I muscle afferents (11 exp.) 76 could be antidromically stimulated from the anterior cerebellum. It should not be assumed that the axons which could not be activated antidromically from cerebellum did not belong to DSCT. The reason is that the rostral part of the anterior cerebellum, which is in apposition to the posterior corpora quadrigemina was not exposed for stimulation and neither was the posterior lobe. Presumably relatively few of the axons terminating in this region could be activated by a stimulus applied to the exposed surface (cf. methods). On the other hand, if an axon could be stimulated in the 5th lumbar segment it could definitely be classified as not belonging to DSCT. Three non DSCT

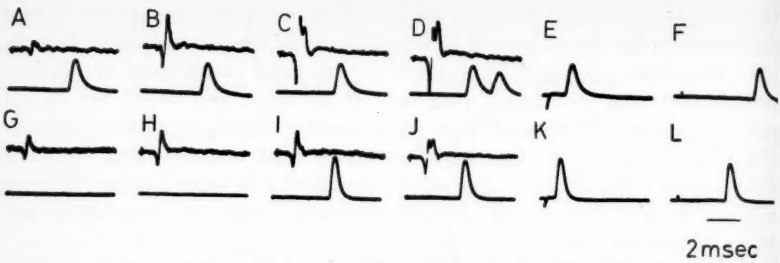

Fig. 2. Microelectrode recording from DSCT axons (lower traces) and recording from the surface of the dorsal column in L5 (upper traces). The left hamstring nerve was stimulated at increasing strength in A—D and G—J. In the unit of records A—F a single spike was evoked by I a volley (record A) and another spike when the stimulus strength was raised to activate group II fibres in D. In the unit of records G—L there was no effect by the maximal group Ia volley in H but when the strength was increased to evoke the small Ib volley in I excitation occurred. Antidromic stimulation from Th8 in E and K and from the cerebellar cortex in F and L. Distance from the site of microelectrode recording to: stimulating electrode in Th8 4.8 cm, anterior cerebellum 22.2 cm.

pathways have been identified in this manner (LUNDBERG and OSCARSSON 1960), but not a single of these axons could be excited by group I muscle afferents.

It has previously been assumed that the neurones activated by group Ia and II as well as those activated by group Ib belong to the DSCT. This has now been proved as illustrated in Fig. 2. In several animals the group I volley displayed perfect separation in Ia and Ib components (BRADLEY and ECCLES 1953, ECCLES, ECCLES and LUNDBERG 1957, LAPORTE and BESSOU 1957). Neurones were identified which could be activated by group Ia and II (Fig. 2, A—D) and others were only activated by impulses in Ib fibres (G—J). With either group the axons could be antidromically stimulated from the cerebellar cortex (record F resp. L).

3. *Neurones activated by the flexion reflex afferents*

In the dorsal part of the lateral funiculus ascends a fairly large number of axons belonging to neurones activated by group II and III muscle afferents, by high threshold joint and by cutaneous afferents (LAPORTE *et al.* 1956 a, b, HOLMQVIST *et al.* 1956, OSCARSSON 1958). The records in Fig. 3 were obtained from such a unit. This neurone received excitation by impulses in high threshold afferents from ipsilateral muscles (A—C), from all the ipsilateral cutaneous nerves tested (D, F and G) and from the mixed tibial nerve (E) but not from the contralateral hamstring nerve (H). The antidromic activation on stimulation of the cerebellar cortex is shown in I and from stimulation of the intact lateral funicle in the mid thoracic region (K). The fibre could not be stimulated in the upper fifth lumbar segment; the latency of the spikes in J show that they are synaptically evoked. Hence it is clear that also units of this type belong to the DSCT. This could not be assumed previously, because

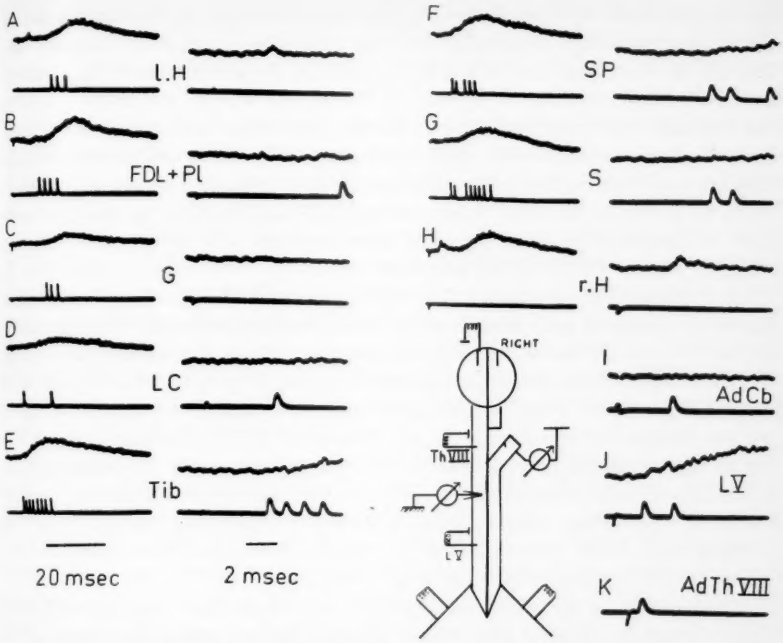

Fig. 3. Lower traces in each records are from a DSCT axon and the upper traces show the mass discharge recorded in the contralateral spinal half. The left and right traces in each record were taken simultaneously at different speeds. Records A—H show the effect of supramaximal stimulation of the nerve indicated in each record. Abbreviations: l. H, left hamstring; FDL + Pl, flexor digitorum longus and plantaris; G, gastrocnemius-soleus; L C, lateral cutaneous; Tib, tibialis; SP, superficial peroneal; S, sural; r H, right hamstring. Antidromic stimulation of the fibre from cerebellum and Th8 is shown in I and K. The discharge in J on stimulation of the lateral funicle in L 5 is due to synaptic activation of the unit. Distance from the site of microelectrode recording to: Stimulating electrode in Th8 7.3 cm, stimulating electrode in L5 4.3 cm, anterior cerebellum 24.7 cm.

in records of the evoked cerebellar potential (LAPORTE *et al.* 1956 a), no potential was found, which corresponded to the late component of the mass discharge caused by activity in these particular axons. This is illustrated in Fig. 5, A and C. The hamstring nerve was stimulated supramaximally in A with optimal recording from the anterior cerebellum and in C, after section of the spinal cord, recording was made from the dissected Flechsig's fasciculus. There is no correspondence in the cerebellar recording to the late component of the mass discharge in C. One of the reasons for this is that the cerebellar potentials were recorded in a decerebrate animal and in this state the interneurones transmitting effects from high threshold muscle afferents to ascending pathways are inhibited from supraspinal centres (HOLMQVIST, LUNDBERG and OSCARSSON 1960).

For interpretation of the present experiments it is important to remember that the descending pathways responsible for this inhibitory control are located bilaterally in the dorsal part of the lateral funicle. To permit antidromic identification from cerebellum the ipsilateral left lateral funicle was intact whereas the contralateral spinal half was sectioned. Hence the supraspinal inhibitory control of the interneurones could exert part of its effect and prevent transmission from the flexion reflex afferents to these neurones to occur as effectively as in the spinal state. In special experiments described in the next section the ipsilateral descending controlling pathway was destroyed while the majority of the DSCT fibres remained intact.

In 8 experiments we found 76 units which could be activated both from cutaneous afferents and from high threshold muscle afferents. Of these units 42, or 55 %, could be antidromically stimulated from the cerebellar cortex. This is a significantly lower percentage than the 76 % found for the group I activated neurones. There is indeed evidence that part of the axons activated by the flexion reflex afferents do not belong to DSCT, because half of the axons not antidromically activated from cerebellum could be stimulated in L5 below the caudal level of Clarke's column, and hence belong to another pathway (LUNDBERG and OSCARSSON 1960). Furthermore, intracellular recording from tract cells activated by these afferents has been made in the lower lumbar segments (ECCLES *et al.* 1960).

The neurones described in this section receive their most effective excitation from cutaneous nerves. A train of 10—20 impulses in response to a single volley in a cutaneous nerve was not unusual. In Fig. 3 the latencies of the first spike evoked from the superficial peroneal (F), the lateral cutaneous (D) and the tibial (E) nerves are so short that excitation of the tract neurones must have been monosynaptic. About 70 % of these units did receive monosynaptic excitation from the sural, the lateral cutaneous, the superficial peroneal or the tibial nerve. 30 % of them received only from one of these 4 nerves whereas the remaining 40 % received monosynaptic excitation from more than one of the nerves tested. However, in the majority of neurones all of the 4 nerves were active in providing excitation to these neurones but in a number of cases the latency of the first action potential was so long in relation to the strength of stimulation that transmission through interneurones was indicated. Hence our findings suggest that cutaneous afferents exert excitatory action both by mono- and polysynaptic pathways.

Adequate excitation of cutaneous receptors was also investigated on neurones of this subdivision of DSCT. The cutaneous nerves were dissected for electrical stimulation but left in intact connection with the receptors to permit adequate activation as well. An extreme amount of variation in the adequate activation from skin were found among these neurones. Some of them were strongly activated by light touch from a rather restricted area which could be as small as two toes or 4 cm^2 on the lateral side of the foot. Others had a larger re-

ceptive field; some could be touch activated from half the hindlimb and one neurone even from the complete hindlimb and part of the belly. Approximately 50 % of the cells were not activated by touch but only by pressure from a receptive field that could be rather restricted or comprise the entire hindlimb. Other units could not even be activated by pressure but some activation was then usually found when the skin was pinched. It was, however, regularly observed in neurones activated by touch that additional activation occurred on pressure and pinching and quite often the receptive field for these latter effects were larger than for touch. Also the intensity of excitation varied a great deal. In many neurones frequencies above 500 per second were found on touch, in a few hard pinching gave only a few spikes. Quite often a considerable after-discharge was noted after cessation of skin stimulation. Inhibitory actions from skin were found in some of these neurones. Only exceptionally did the inhibitory area surround the excitatory one; it was often remotely situated and had no predictable spatial relationship to the excitatory area.

In summary these neurones constitute a very heterogenous group with respect to their adequate activation from skin. One characteristic feature is the convergence of different cutaneous modalities as evidenced by activation on touch, pressure and pinching. Now it should be mentioned that units were included in the group described in this section if excitation was evoked from any of the muscle nerves tested, hamstring, triceps surae, flexor digitorum longus and plantaris *i. e.* even if only a single spike resulted on supramaximal stimulation of one of these nerves. Such a weak excitation probably is of very doubtful significance (cf ECCLES, FATT and LANDGREN 1956, ECCLES, ECCLES and LUNDBERG 1960) and possibly such neurones should be classified with those described in the next section which receive excitation only from cutaneous afferents. There was a tendency for units with very strong excitation from muscles (6—10 spikes on supramaximal single volley stimulation of muscle nerves) to have larger cutaneous receptive field and several of the neurones with very weak excitation from muscle had small cutaneous receptive field. However there was no absolute correlations, in a few of the neurones with strong excitation from muscle quite restricted cutaneous receptive fields were found.

4. *Neurones activated exclusively by cutaneous afferents*

It was noted early during the course of this investigation that some neurones identified by antidromic activation as belonging to DSCT could be orthodromically activated only by volleys in cutaneous nerves and not on stimulation of muscle nerves. At first we suspected that they belonged to the group of neurones described in the preceding section, *i. e.* those activated by the flexion reflex afferents but that transmission of the effects from high threshold muscle afferents were prevented by the descending inhibitory control in the

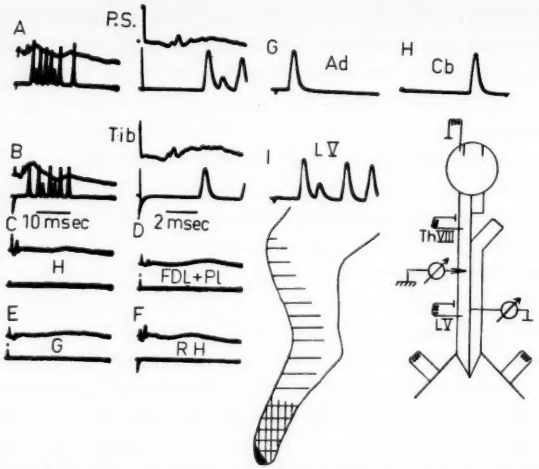

Fig. 4. As in Fig. 3. The right and left traces in A and B were obtained simultaneously at different speeds. C—F were obtained at slow speed, G—I at the fast speed. Abbreviations as in Fig. 3. Observe that activation from L5 in record I is synaptic. In the drawing of the hindlimb the receptive fields of this unit are shown: the small black area is the field for light touch, the vertically hatched area for pressure and the horizontally hatched area for pinching. Distance from the site of microelectrode recording to: stimulating electrode in L5 5.8 cm, stimulating electrode in Th8 5.8 cm, anterior cerebellum 22.8 cm.

intact ipsilateral lateral funicle. We have now, by the following type of experiment, satisfied ourselves that there is a pure "skin" subdivision of DSCT not excited by high threshold muscle afferents when the inhibitory supraspinal control of interneurones has been removed. For this purpose a lesion was made in the dorsal half of the lateral funicle sparing only a superficial lateral strip containing many DSCT fibres. To control the effect of the lesion the mass discharge was recorded from contralateral ascending spinal pathways, which are controlled in a similar way from supraspinal centres (HOLMQVIST *et al.* 1960). After the lesion there was a large increase of the discharge in the contralateral spinal half. On subsequent complete sectioning of the cord there was no further release of mass discharge in the contralateral spinal half; hence the supraspinal control had been effectively eliminated and DSCT axons at the same time been left in intact connection with cerebellum to permit antidromic identification of them.

Fig. 4 shows such a unit. It could not be activated by supramaximal stimulation of any of the muscle nerves (C—F) but a train was evoked by a single volley from the superficial peroneal nerve and the tibial nerve, the latency in both cases being so short as to indicate monosynaptic transmission for the first spike. Antidromic excitation from cerebellum is shown in H and from the midthoracic region in G. The receptive field for adequate skin excitation is shown

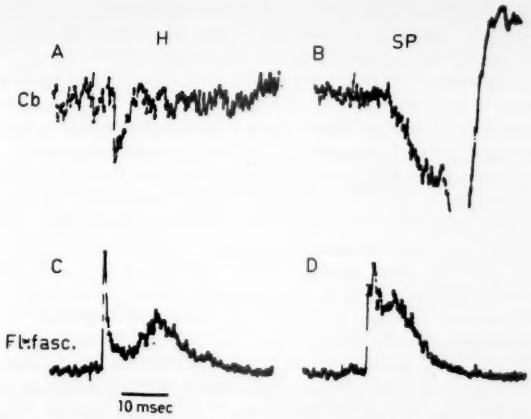

Fig. 5. Supramaximal stimulation of the left hamstring nerve in A and C and of the left superficial peroneal nerve at the ankle in B and D. The evoked potential in A and B were recorded from the left anterior cerebellum with a wick electrode. The indifferent electrode was placed in the temporal muscle. C and D were recorded in the same experiment from the left dissected Flechsig's fasciculus in the lower thoracic region after section of the spinal cord.

in the drawing of Fig. 4. It discharged intensely on movement of hairs from a restricted area between and on the dorsum of the toes, in addition it was very effectively excited by light pressure from the area hatched with vertical bars and finally by pinching from the larger horizontally hatched area in the drawing. The units of this group in their mode of adequate activation from skin reminded of those described in the last section in so far as they are also activated by touch, pressure and pinching. Likewise there was a considerable variability in the receptive fields from which these actions were drawn. In many the receptive field for touch was small as in Fig. 4, but in others it was larger the maximum being the thigh or approximately about one half of the hindlimb. None of these units could be activated from the skin of the entire hindlimb as was the case with a few described in the preceding section. These units and those described in the preceding section were never excited by pressure on the pads.

Evoked cerebellar potentials

The majority of the DSCT neurones described in this and the preceding section are monosynaptically activated by impulses in cutaneous afferents. It is pertinent to consider this finding in relation to investigations on evoked cerebellar potentials (cf. Dow and Moruzzi, 1958). On recording from the anterior cerebellum Grundfest and Campbell (1942) found a potential sequence of three early components. The two early components were abolished after transection of the ipsilateral Flechsig's fasciculus. Mountcastle, Covian and Harrison (1952) and Laporte *et al.* (1956 a) confirmed these findings and demonstrated that the second component was evoked on stimula-

tion of skin nerves. Component I was evoked on stimulation of group I muscle afferents and corresponded to the discharge recorded in Flechsig's fasciculus on stimulation of these afferents. OSCARSSON (1956) has shown that the discharge in VSCT fibres also contributes to component I of the evoked cerebellar potential and this component can only be correlated with the discharge in Flechsig's fasciculus if the contralateral VSCT has been interrupted. On the other hand there was no equivalence between the second component and the discharge resulting in Flechsig's fasciculus on stimulation of cutaneous afferents. This is shown in Fig. 5 with recording from cerebellum in B and from the dissected Flechsig's fasciculus in D. There is no correspondence in the cerebellar discharge to the well synchronized early potential recorded from Flechsig's fasciculus, which is monosynaptically transmitted from cutaneous afferents as shown by LAPORTE *et al.* (1956). Hence it could not be assumed that the fibres conducting this well synchronized cutaneous volley were part of DSCT. In a succeeding paper (LUNDBERG and OSCARSSON, 1960) it will be further demonstrated that this discharge is caused by impulses in a pathway not belonging to DSCT (cf also WALL, 1960, ECCLES, ECCLES and LUNDBERG, 1960). In fact these pathways have anatomically separate locations in the cord, the pathway referred to is located medially of the DSCT (LUNDBERG and OSCARSSON, 1960). Nevertheless it is now clear that many DSCT neurones are monosynaptically activated by cutaneous afferents, and the question may be raised if the earliest part of component II in the cerebellar potential is not evoked by impulses in these cerebellar afferents, in the same way as component I represents the activity in group I activated DSCT fibres. Fig. 5 demonstrates that this is the case. The difference in latency in A and C is 2.7 msec the corresponding difference in B and D is 3.0 msec. It is known that in the lower thoracic region the cutaneous non-DSCT discharge precedes the cutaneous DSCT discharge by 0.3 msec (LUNDBERG and OSCARSSON, 1960); hence it can be concluded that the early part of the deflection in B must represent activity in DSCT fibres monosynaptically activated by cutaneous afferents.

5. *Neurones activated by pressure on pads*

In experiments with adequate activation it was noted by LAPORTE and LUNDBERG (1956) that many axons in the dorsal part of the lateral funicle responded with a high frequency discharge on pressure of pads. We have now found that the DSCT also has a pad-subdivision. These units always had monosynaptic connection from large afferents in the tibial nerve and responded to a single volley with a high frequency train of 3—10 impulses. Usually they received excitation from the superficial peroneal nerve and the lateral cutaneous nerve as well. Some of the pad units were excited also from muscle afferents. Of 12 units one was excited by a group I volley from gastrocnemius-soleus and 4 received weak excitation from high threshold muscle afferents. With adequate stimulation the receptive field was one or two pads, but in some cases only part of one pad, particularly with units activated from the larger central pad. They were activated by light pressure and with maintained pressure there was only moderate adaptation. Very high frequencies were often attained as in the case illustrated by LAPORTE and LUNDBERG (Fig. 9, 1956) with 700/sec initially during strong pressure. The typical pad unit could not be activated from the skin surrounding the pad, only in one case was it

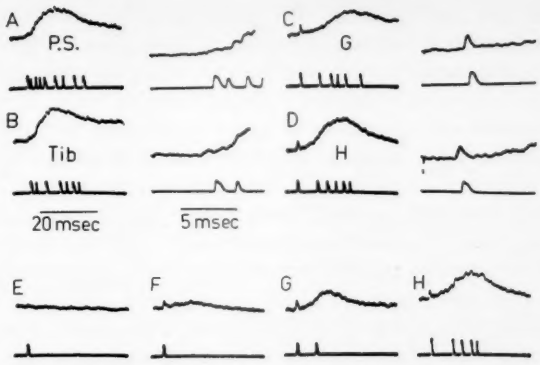

Fig. 6. As in Fig. 3. Simultaneous recording at two speeds in A—D. Record E—H were obtained at the slow speed with stimulation of the hamstring nerve at increasing strengths. The strength in E was at threshold for evoking the VSCT discharge in the contralateral spinal half. The later spikes resulted when the stimulus strength had been increased considerably so as to evoke the later mass discharges in the upper trace of G and H. The unit was identified by antidromic stimulation from the anterior cerebellum but was lost before any records had been taken.

not possible to exclude a subsidiary effect from a few hairs close to the pad. Like all other subgroups of DSCT these units were spontaneously active, sometimes they received some inhibitory action on bending of the ankle joint (cf LAPORTE and LUNDBERG 1956).

6. *Other types of DSCT units*

With units excited by electrical stimulation of the mixed tibial nerve classification depended on the mode of adequate activation. It was usually not difficult to differentiate units activated by pressure of skin from those activated from foot muscles. The latter units could be activated by bending of joints and in addition by gentle manipulation of a muscle belly. Some units were found which responded to bending of joints but not from any muscle. We suspect that these are activated by joint afferents and it cannot be excluded that these units represent a sixth subdivision of the DSCT. We prefer, however, to leave this question open; this problem must be specially investigated preferably with the usage of the knee joint with well known receptors and afferents (cf. SKOGLUND 1956).

A number of other DSCT units have been found which could not be grouped into any of the 5 subdivisions described above or rather seem to represent intermixtures between them. For example, some units were found which, in addition of being activated from group I muscle afferents, received excitation also from cutaneous afferents and from high threshold muscle afferents as illustrated in Fig. 6. A train of impulses was evoked on stimulation of the super-

ficial peroneal (A), and the tibial (B) nerves; in both cases the latency of the first spike is so brief as to indicate monosynaptic transmission. On supramaximal stimulation of the hamstring (D) and the gastrocnemius-soleus (C) nerves the unit responded with a short-latency spike followed by train. In each case the first spike resulted on stimulation of group I afferents and the later trains with activation of high threshold afferents. This is illustrated in E—H with increasing strength of stimulation of the hamstring nerve. The first spike appears at group I strength giving a barely visible VSCT mass discharge in the contralateral spinal half (caused by impulses in Ib afferents, OSCARSSON 1957). In G and H the stimulus strength was raised to excite high threshold afferents causing the late mass discharge in the contralateral half (OSCARSSON 1958). It was reported by LAPORTE *et al.* (1956) that a few group I activated units could be excited from cutaneous nerves. In the present series only few group I activated units received such a strong activation from cutaneous and high threshold muscle afferents as in Fig. 6 but 13 % received some excitatory effects from high threshold muscle afferents or from skin afferents.

It is known that group I activated units can be inhibited by group I impulses from antagonist and other muscles (LAPORTE *et al.* 1956 b, LUNDBERG and OSCARSSON 1956, HOLMQVIST *et al.* 1956, CURTIS, ECCLES and LUNDBERG 1958). In the present experiments some group I activated units were found which in addition received inhibitory action from cutaneous afferents and from high threshold muscle afferents. This confirms the finding that the group I evoked mass discharge in Flechsig's fasciculus can be somewhat inhibited by a conditioning volley in cutaneous or high threshold muscle afferents (OSCARSSON 1957) an effect which although weaker is reminiscent of the effect on the VSCT (OSCARSSON 1957).

7. *Termination of DSCT axons*

Antidromic activation of DSCT fibres offers an opportunity to investigate the termination of this pathway in cerebellum. There has been agreement between anatomical investigators that the bulk of the dorsal spino-cerebellar fibres terminate ipsilaterally in the anterior lobe, but some fibres reach the pyramis and its adjoining folia (MACNALTY and HORSLEY 1909, INGVAR 1918, BECK 1927, BRODAL and JANSEN 1941, ANDERSON 1943). We have been concerned mainly with the lateral extension of the terminal area in the anterior lobe. The map in Fig. 7 refers to culmen, neither lingula and lobulus centralis of the anterior lobe nor pyramis, uvula and the tuber were exposed for stimulation. To decide the termination of a fibre a slightly supraliminal stimulus strength was used and the cortical surface explored with the stimulating electrode. When the area from which the fibre could be activated at low stimulus strength was small and surrounded by cortex from which no antidromic activation was obtained, it was assumed that this area represents the cortical termination of the fibre. A number of fibres were activated at higher strengths

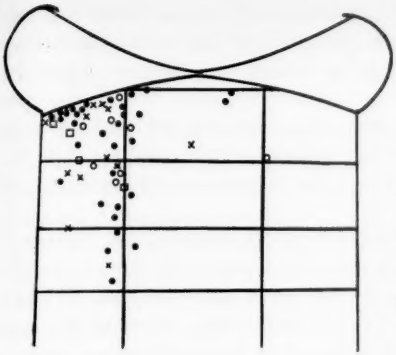

Fig. 7. Termination of 57 DSCT axons as indicated by the low threshold foci for antidromic activation of individual axons. Vertical lines denote borders of the intermediate regions, horizontal lines sulci. The different signs denote activation from primary afferents as follows: (●) group I muscle afferents, (×) cutaneous and high threshold muscle afferents, (○) cutaneous afferents, (□) pad afferents.

from the anterior margin of the exposed cortex. Presumably these fibres terminated in lobulus centralis. There was never evidence of multiple termination of DSCT fibres. By contrast the axons of the ventral spino-cerebellar tract could usually be stimulated from large areas and there was often evidence that an individual axon had two independent terminal areas (LUNDBERG and OSCARSSON unpublished). Hence we infer that the individual DSCT axons supply only a small cortical area. Fig. 7 shows that the bulk of the DSCT fibres terminate in the intermediate region of the anterior lobe and not in the vermis proper. However a few DSCT axons cross and terminate in the contralateral anterior cerebellum as is shown in Fig. 7. In this connection it should be remembered that the large majority of DSCT axons do not cross in the spinal cord. In our material there was only one crossed unit (monosynaptically activated by group I and II impulses from the contralateral hamstring nerve), previously one has been found by OSCARSSON (1957).

Discussion

It must now be recognized that the functional organization of the dorsal spino-cerebellar tract is considerably more complicated than had hitherto been assumed in so far as it contains at least 5 functional subdivisions.

In addition to the two previously recognized proprioceptive pathways giving muscle spindle respectively Golgi tendon organ information, there are two subdivisions activated exclusively by afferents from exteroceptors. One of these channels informs about pressure on pads and each neurone usually is activated only from one or part of one pad. It is not difficult to understand that a message of this type is of importance in cerebellar integration of posture and movement. Neurones of the other exteroceptive channel are activated by slight touch,

pressure and pinching of a smaller or large cutaneous area. Absence of modality specificity is characteristic, but does not exclude interpretation in terms of modalities since individual neurones are activated to a different degree by these modes of skin stimulation. Also some neurones may have a small receptive field for slight touch and a much larger for pressure and for nociceptive skin stimulation. In any case the anterior cerebellum through this DSCT channel gets reasonably good spatial information concerning cutaneous events.

The fifth DSCT subdivision has a more complex organization because it can neither be classified as exteroceptive nor as proprioceptive. These neurones are activated by what we have called the flexion reflex afferents: group II and III muscle afferents, high threshold joint afferents and cutaneous afferents. A number of ascending pathways are influenced by these afferents (LAPORTE *et al.* 1956 b, HOLMQVIST *et al.* 1956, OSCARSSON 1957, 1958). Their functional significance was discussed by HOLMQVIST *et al.* (1960) in relation to the finding that transmission to them is controlled from supraspinal centres through inhibition of interneurones in the same way as is the case with the flexion reflex actions (ECCLES and LUNDBERG 1959, HOLMQVIST and LUNDBERG 1959). It has been shown that in spinal animals units of this type can be effectively activated from muscle (HOLMQVIST *et al.* 1956) but it is noteworthy that the dominating excitatory action to this DSCT subgroup usually is from skin and that skin afferents have monosynaptic connections with the majority of these cells. In their mode of adequate activation from skin these neurones remind of the units of the fourth DSCT-subgroup, which are excited exclusively from skin afferents. They are adequately excited both by touch, pressure and pinching of skin but sometimes the receptive fields were larger, particularly for neurones with strong excitation from muscle afferents. In a forthcoming report on the supraspinal control of transmission to DSCT it will be shown that when the supraspinal controlling system of transmission from the flexion reflex afferents is activated, the actions from muscle afferents are suppressed whereas the effects from skin usually escapes the control (LUNDBERG and OSCARSSON, unpublished). Hence this DSCT channel when controlled by supraspinal centres may function as a pure exteroceptive pathway.

A relatively small number of DSCT neurones could not be classified as belonging to any of the 5 subdivisions discussed above or seemed to represent intermixtures. It was observed that some units activated by group I afferents, in addition to the previously described group I inhibition (LAPORTE *et al.* 1956, LUNDBERG and OSCARSSON 1956, HOLMQUIST *et al.* 1956, CURTIS *et al.* 1958), also receive inhibitory action by the flexion reflex afferents (cf. also OSCARSSON 1957). Other group I activated units sometimes receive weak excitation by the flexion reflex afferents (cf also LAPORTE *et al.* 1956 b). One of the pad units could be excited by impulses in group I

afferents from the gastrocnemius-soleus nerve and some received weak excitation from high threshold muscle afferents. It is impossible to say if these more exceptional connections represent integration at the second order neurone level or if these connections merely should be classified as aberrant without much functional significance among the five dominating subdivisions.

These experiments have also given information on the termination of the DSCT. Exact knowledge of the termination of spino-cerebellar tracts is of particular importance because of the recent disclosure that cerebellum is organized into longitudinal cortico-nuclear zones as opposite to the earlier lobar concept (JANSEN and BRODAL 1940, JANSEN 1954, CHAMBERS and SPRAGUE 1955 a, b, MORUZZI and POMPEIANO 1957, BATINI and POMPEIANO 1958, POMPEIANO 1958, DOW and MORUZZI 1958). The anterior lobe of cat has one vermal part projecting to fastigius and one intermediate part projecting to interpositus but the lateral part is lacking. Our findings indicate that the bulk of DSCT fibres project to the ipsilateral intermediate cortex. Most anatomical investigators state that the DSCT ends in the vermis of the anterior lobe but there is no natural borderline between vermis and the intermediate cortex and it seems probable that in these accounts vermis included the intermediate part (cf BRODAL 1954). Actually it has been emphasized by BECK (1927) and ANDERSSON (1943) that the DSCT ends quite laterally and in a human case BRODAL and JANSEN (1941) were able to establish that the DSCT at least partly terminates in the intermediate cortex. POMPEIANO (1958) has shown that the intermediate cortex can be functionally divided into one medial and one lateral strip with different subcortical connections. The DSCT seems to terminate both in the paravermian and the remaining lateral part.

As to the general role of the information forwarded by the DSCT it is obviously of interest to learn about the function of the intermediate cortex. An indication may be given by the findings of CHAMBERS and SPRAGUE (1955 a) who observed the behaviour of animals after total lateral lesions of the interposite nucleus:

> These animals "showed postural defects limited to the ipsilateral limbs. These consisted of mild but readily noticeable increase in extensor tone and in the supporting reflex. The tactile placing reflex was permanently abolished, and proprioceptive placing and hopping were absent or sluggish during the first week and remained permanently hypermetric and easily fatigued. Both ipsilateral limbs showed goosestepping, and frequently assumed bizarre positions at rest without correction throughout the survival period. The ipsilateral foreleg was stiff and hypermetric in visual placing, and it was clumsy and poorly aimed in batting and striking movements. Impairment of movement of the ipsilateral foreleg was seen easily when the animal was held for testing the placing reactions: this limb was held extended and immobile, and seldom used, as was the normal leg in an attempt to dislodge the observer's hand. During the first 7—10 days when proprioceptive reflexes were absent or very poor, the animal frequently came to rest standing on the dorsum of the forefoot. Both ipsilateral feet were dragged in walking throughout the survival period with instability at wrists and ankles, and showed

persistently poor placing when walking on bars. This deficit showed itself in its extreme form in repeated oscillations of the feet, for in each attempted placement the foot would swing forward, drag across the bar, overreach it, retract again across the bar and overreach it again. This rhythm was often maintained for many seconds. Visual attention by the animal did not repair this deficit."

By contrast, after total unilateral fastigial lesions the same authors found marked postural deficits involving the entire body-head, trunk, limbs and tail. These differences are clearly of interest in the present context even if our lack of knowledge of cerebellar interconnections makes it difficult to imagine what functional significance should be attached to regional cerebellar functions (cf Dow and Moruzzi 1958).

Summary

Recording was made from single fibres in Flechsig's fasciculus and axons were identified as belonging to the dorsal spino-cerebellar tract when they could be antidromically activated from the cerebellar cortex.

It is assumed that a fibre terminates in the cortical region from which the axon can be activated at threshold stimulation. The bulk of dorsal spino-cerebellar tract fibres terminates ipsilaterally in the intermediate cortex of the anterior lobe.

Conduction velocities in dorsal spino-cerebellar fibres range from about 100—30 m/sec.

Five main functional subdivisions of the dorsal spinocerebellar tract could be distinguished on the basis of sensory input:

1) Neurones monosynaptically activated by impulses in muscle spindle afferents (Ia + II).

2) Neurones monosynaptically activated by impulses in Golgi tendon organ afferents.

1) and 2) were previously assumed to belong to the dorsal spino-cerebellar tract.

3) Neurones activated exclusively by light pressure on pads usually from one or two pads but sometimes only from a small area of one pad.

4) Neurones activated exclusively from skin. Many units of this group were effectively activated by tactile stimuli from a relatively restricted cutaneous area, but they received additional activation on pressure and pinching and these actions were usually drawn from a larger receptive field.

5) Neurones activated from skin as 4) and in addition by high threshold muscle afferents. With some of these units the cutaneous receptive fields were very large.

Intermixtures between some of these groups have been found.

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A Study of the Anaphylactic Mast-Cell Reaction in vivo Following Desensitization of Sensitized Guinea Pigs

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Abstract

BORÉUS, L. O. *A study of the anaphylactic mast-cell reaction in vivo following desensitization of sensitized guinea pigs.* Acta physiol. scand. 1960. *50.* 375—384. — The sensitivity of mast cells to antigen was studied quantitatively in the nasal mucosa of sensitized guinea pigs under pentobarbital anaesthesia. The typical anaphylactic mast-cell "disappearance", provoked by i. a. challenge injections of the antigen, could be inhibited with preceding s.c. antigen injections. This desensitization of mast cells was found to have developed within 1 hour following an appropriate s.c. injection and to last for about 2 weeks. Its effectiveness was proportional to the size of the preceding dose of the antigen. The desensitization was never absolute, since high challenge doses could still produce some degree of mast-cell disappearance. Injections of antigen during the period of sensitization postponed the development of mast-cell sensitivity to the antigen. Weekly injections of the antigen could maintain the desensitized state. Desensitization with one antigen (egg albumin) did not influence the sensitivity of the mast cells to another antigen (horse serum) and *vice versa* in double-sensitized animals. In all experiments the inhibition of mast-cell reactivity to antigen corresponded to an inhibition of the general anaphylactic symptoms.

It is well known that sublethal doses of antigen make sensitized animals temporarily resistant to subsequent antigen administration. This desensitization is generally considered to be due to saturation of tissue antibodies by the antigen, so that further antigen administration is ineffective.

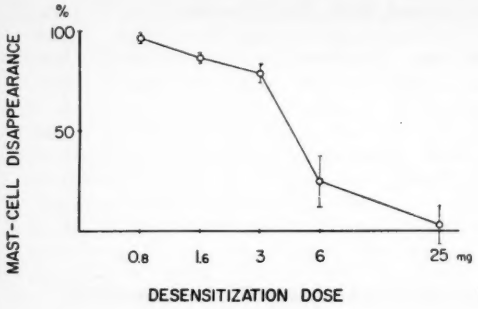

Fig. 1. Influence of the desensitization dose on the anaphylactic mast-cell disappearance provoked one day after administration of the dose by challenge with 100 mg egg albumin. Mean values and standard errors of 4—6 disappearance values.

In an earlier communication (BORÉUS 1960) the anaphylactic mast-cell reaction (the "mast-cell disappearance") was studied quantitatively in the nasal mucosa of living, anaesthetized guinea pigs. Since the mast-cell disappearance was found to be correlated to the challenge dose of antigen, it seemed worthwhile investigating the role of this cell reaction following desensitization of the animals.

Methods

Guinea pigs of both sexes were used.

Sensitization was produced by a s.c. injection of 100 mg of crystalline egg albumin in 1 ml of a 0.9 % w/v NaCl solution, followed 3 days later by the same dose, given i.p. In the experiments with doubly-sensitized guinea pigs, injections of 1 ml of horse serum were given at the same time.

Desensitization was performed with s.c. injections of antigen in unanaesthetized animals.

Anaphylaxis was provoked in the anaesthetized guinea pig (pentobarbital about 40 mg/kg i.p.) 2—6 weeks after sensitization. Cannulas were inserted into the trachea and into the cranial end of the common carotid artery. The bone covering the nasal cavity was removed and two control specimens were taken from the inferior nasal concha on the one side. After i. a. injection of the challenge dose of antigen (injection volume 1 ml, injection time 1 min), the intensity of the general anaphylactic symptoms was noted (various degrees of distressed respiration with vigorous inspiratory movements, gasping, cyanosis, defaecation, urination etc., death in severe cases in a few minutes); 4 min after the injection, two test specimens were taken from the corresponding part of the contralateral concha.

After fixation and sectioning, the number of recognizable mast cells per square unit was estimated for each specimen. The difference between a test specimen and its corresponding control specimen was expressed as a percentage decrease from the control and called "the mast-cell disappearance". The details of this method have been described earlier (BORÉUS 1960).

Results

Different desensitizing doses. 12 sensitized guinea pigs were given different desensitizing doses of antigen. Next day, the mast-cell reactivity was estimated by giving a standard challenge dose of 100 mg egg albumin. The results are shown in Fig. 1. The smaller doses (0.8—3 mg) did not significantly depress

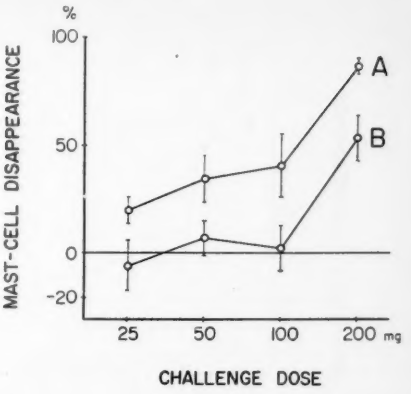

Fig. 2. Influence of the challenge dose on the anaphylactic mast-cell disappearance provoked after s.c. desensitization with (A) 5 injections of 1 mg egg albumin each, given singly every other day during 9 days (challenge dose given on day 10) and with (B) one single injection of 25 mg egg albumin (challenge dose given one day later). Mean values and standard errors of 5—8 disappearance values.

the mast-cell reaction to the antigen, but 6 mg and 25 mg produced desensitization. The intensity of the anaphylactic shock symptoms corresponded to the disappearance values. Thus, one of the three animals which had received 6 mg on the preceding day died of anaphylactic shock, whereas none of the 25 mg animals died. All animals with desensitizing doses of less than 6 mg died of anaphylactic shock when given the challenge dose.

The desensitizing injections themselves produced no symptoms when the dose was 0.8 mg or 1.6 mg. Doses of 3 mg and more caused increasing degrees of restlessness and weakness in most animals. Desensitizing doses of more than 25 mg were not given, since preliminary experiments had shown that they caused death in most animals after a few hours.

The influence of the desensitizing dose on the absolute mast-cell population of the nasal mucosa is discussed below.

Different challenge doses. In order to determine the significance of the size of the challenge dose, two groups of sensitized guinea pigs were desensitized and then given different challenge doses of the antigen (25, 50, 100 and 200 mg egg albumin).

In the first group (13 guinea pigs) the desensitization was produced by a single 25 mg injection (challenge dose given on the following day) and in the second group (15 guinea pigs) by five s. c. injections of 1 mg each, given every second day during a 9-day period (challenge dose given on the 10th day).

The results of these experiments are summarized in Fig. 2. The single injection gave a slightly more effective desensitization than did the five injections of a smaller amount of antigen (P between 0.2 and 0.05 for the 25, 50 and 100 mg doses, P between 0.05 and 0.01 for the 200 mg dose). In both cases, however, the mast cells still reacted with disappearance if a very high challenge dose was administered. On the whole, the general anaphylactic symptoms parallelled the intensity of the mast-cell reaction.

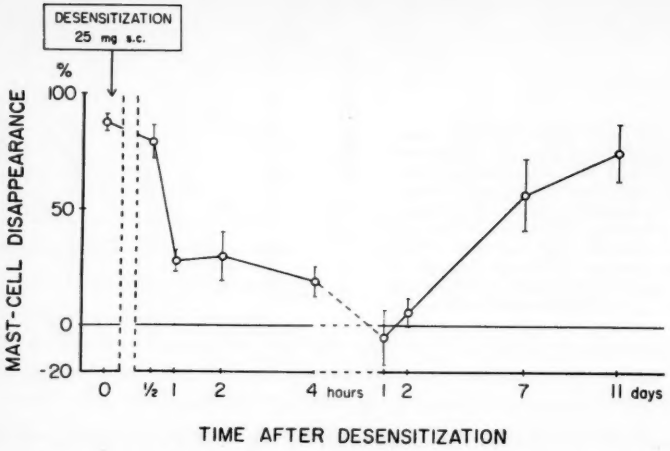

Fig. 3. Time curve showing induction time and duration of the desensitized state of guinea-pig mast cells. Desensitization produced by a single s.c. injection of 25 mg egg albumin. Challenge dose 25 mg egg albumin. Mean values and standard errors of 4—8 disappearance values.

In most cases, the 25 mg desensitizing dose of the antigen produced shock symptoms *per se* (weakness, dyspnoea) and 7 animals out of the original 20 died after a few hours; the remaining 13 had recovered by the next day. No shock symptoms were ever observed in the second group, which received 1 mg at each injection.

The influence of the desensitizing process on the absolute mast-cell population in the nasal mucosa could be elicited by comparing the cell counts in the control specimens (taken before the challenge dose was given) in the two desensitized groups. These populations were: 1.84 ± 0.16 cells/square unit (1 square unit = 0.256 mm²) in the 25 mg single injection group, and 2.35 ± 0.12 in the 5×1 mg injection group. The P level of the difference lies between 0.01 and 0.05. Thus, the single injection of 25 mg itself results in some mast-cell disappearance. The same is probably not true for the 5×1 mg doses, because in an earlier series of 100 control specimens (BORÉUS 1960) the mean mast-cell population for 56 animals was 2.41 ± 0.15, and this is not statistically different from the value obtained in the 5×1 mg injection group in this experiment.

Time curves. Time curves for the desensitization process were established by testing the mast-cell reactivity at different intervals after desensitization. The time required to produce the desensitized state was studied by giving the challenge dose (25 mg) $^1/_2$, 1, 2 and 4 hours after a desensitizing dose of 25 mg. Fig. 3 reveals that the mast-cell reactivity is lowered already after 1 hour and

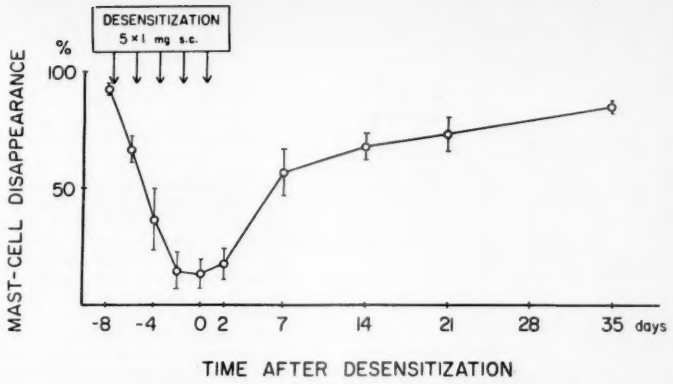

Fig. 4. Time curve showing induction time and duration of the desensitized state of guinea-pig mast cells. Desensitization produced by 5 s.c. injections of 1 mg egg albumin each, one on every second day during a 9-day period. Challenge dose 25 mg egg albumin. Mean values and standard errors of 4—8 disappearance values.

then gradually decreases. However, it subsequently increases again and after 11 days it has almost reached the initial value.

A similar time curve is seen in Fig. 4, where desensitization was performed by means of 5 s. c. injections of 1 mg every second day. The mast-cell reactivity subsequently decreased following the first three injections. On day 7, a definite increase of mast-cell sensitivity was noted, and this then slowly approached the initial value.

Postponement of sensitization by desensitization. It was shown earlier (BORÉUS 1960) that the anaphylactic mast-cell reaction in the guinea pig can not be produced until the 9th day following the first sensitizing injection. Since it is known that the onset of sensitivity, as measured by the anaphylactic shock symptoms, may be postponed by the injection of antigen just prior to the development of hypersensitivity, a series of experiments was performed to determine whether this postponement is reflected in the mast-cell reaction to the antigen.

Fifteen unsensitized guinea pigs were injected on day 0 with 100 mg egg albumin s. c. in order to produce sensitization. The same dose was given i. p. on day 3. On days 6 and 9, 4 animals received 100 mg egg albumin s.c., 4 received 1 mg and 4 received 0.1 mg. As controls, one animal from each of these 3 groups was challenged with 25 mg egg albumin on day 7. None of these 3 animals reacted with any shock symptoms and the mast-cell disappearance was around 0 %, indicating that the hypersensitive state had not yet been established. Two of the remaining animals from each group were challenged on day 11. The disappearance values obtained are shown in Fig. 5.

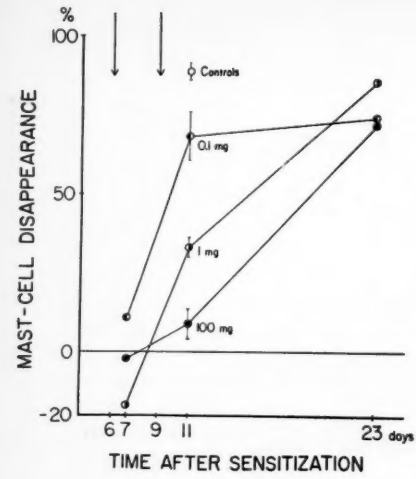

Fig. 5. Postponement of sensitization of guinea-pig mast cells by means of 2 s.c. injections of different doses of egg albumin on days 6 and 9 (indicated by arrows) after the first sensitizing dose. Challenge with 25 mg egg albumin on days 7, 11 and 23. Mean values of 2 disappearance values (days 7 and 23); mean values and standard errors of 4 disappearance values (day 11). "Controls" denotes 6 disappearance values from three animals which had not received the desensitizing injections on days 6 and 9.

The 0.1 mg injections did not significantly prevent the onset of sensitivity, as compared with three control animals, which had received no antigen injections during the sensitization period. However, the animals with the 100 mg injections were still unsensitive to the antigen. The 1 mg injections produced a condition of intermediate sensitivity. The remaining animal of each group was tested on day 23. All three animals then evinced strong mast-cell reaction and fatal anaphylactic shock.

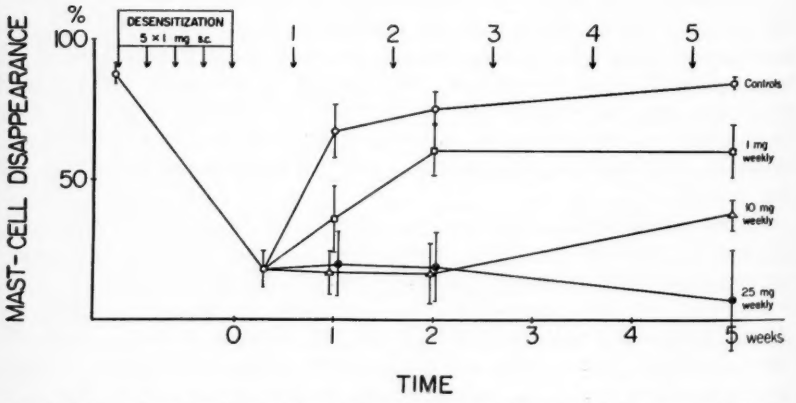

Fig. 6. Effect of prolonged administration of antigen on the anaphylactic mast-cell disappearance. Arrows 1—5 indicate the weekly s.c. injections of egg albumin. Mean values and standard errors of 4—8 disappearance values.

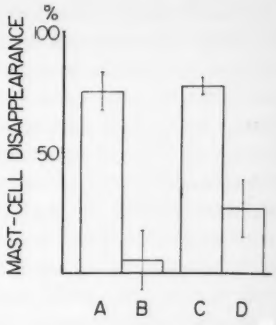

Fig. 7. Effect of s.c. desensitization of double-sensitized guinea pigs on the anaphylactic mast-cell disappearance. Mean values and standard errors of 4 disappearance values. A: Desensitized with 5 × 1 mg egg albumin, challenged with 1.5 ml horse serum. B: Desensitized with 5 × 1 mg egg albumin, challenged with 25 mg egg albumin. C: Desensitized with 5 × 0.1 ml horse serum, challenged with 25 mg egg albumin. D: Desensitized with 5 × 0.1 ml horse serum, challenged with 1.5 ml horse serum.

None of the animals showed any shock symptoms whatsoever when injected with egg albumin on days 6 and 9.

Prolonged administration of antigen. To investigate if the desensitization of mast cells could be maintained by subsequent injections of antigen at regular intervals, 35 sensitized guinea pigs were desensitized by 5 injections of 1 mg egg albumin, one every second day during a 9-day period. 4 of these animals were challenged as controls on the 10th day with 25 mg egg albumin. The other guinea pigs were divided into 4 groups. Group 1 (10 animals) served as controls and received no further antigen injection. Group 2, 3 and 4 (7 animals each) received one s.c. injection once a week for 5 weeks of 1 mg, 10 mg and 25 mg, respectively. 2—3 animals from each group were challenged with 25 mg egg albumin at 1 week, 2 weeks and 5 weeks after desensitization. The values obtained are given in Fig. 6.

It is seen that the desensitization procedure reduced the mast-cell response to antigen from 87.7 ± 3.8 % (untreated controls) to 17.9 ± 6.5 % disappearance. This difference is significant ($P < 0.001$). If no further dose of the antigen is given (control group), the disappearance values rapidly approach the pre-desensitization values. Weekly injections of the antigen, however, maintain the lowered susceptibility of mast cells to it, higher doses being more effective than smaller ones. Thus, a statistical comparison of the 5-week values for mast-cell disappearance in the different groups reveals that the controls differ from the 10 mg and 25 mg groups so that $P < 0.001$ and from the 1 mg group so that $P = 0.05 — 0.01$.

The absolute mast-cell population in the control group after 5 weeks was 2.38 ± 0.25 cells/square unit. In the 1 mg and 10 mg groups the counts were similar, 2.31 ± 0.34 and 2.14 ± 0.29, respectively. In the 25 mg group, however, the corresponding figure was 1.64 ± 0.22, which differs from the control at a P level between 0.05 and 0.01.

The intensity of the general anaphylactic symptoms corresponded to the degree of mast-cell reaction.

Desensitization of double-sensitized animals. A series of experiments was made to see if desensitization of mast cells to one antigen is accompanied by refractoriness to another antigen as well. 8 guinea pigs were sensitized by means of a s.c. injection of 100 mg egg albumin + 1 ml horse serum. This dose was given again 3 days later i.p. Two weeks later, 4 animals were desensitized by means of 5 s.c. injections of 1 mg egg albumin, and 4 animals desensitized by means of 5 s.c. injections of 0.1 ml horse serum. The injections were given on every second day during a 9-day period. On the 10th and 11th days, two animals of each group were challenged with 25 mg egg albumin and two of each group with 1.5 ml of horse serum. The results are given in Fig. 7. It is seen that desensitization against one antigen does not prevent the anaphylactic mast-cell reaction to the other antigen.

Discussion

Sensitized animals, which survive the antigen-induced anaphylactic shock, are resistant to a second dose of the same antigen. This phenomenon, recognized as a "specific desensitization", is generally considered to involve a slow saturation of tissue antibodies, fixed at tissue cells, with antigen until they are no longer receptive to it. This explanation rests on several well-established experimental findings *in vitro*. It is well known that it is possible to saturate anaphylactic antibodies with the specific antigen *in vitro* and that the isolated guinea-pig uterus or ileum, studied with the Schultz-Dale technique, does not contract upon antigen administration after treatment with the specific antigen. In the living animal, however, the lack of suitable techniques has obstructed attempts to demonstrate directly and quantitatively a decreased cell sensitivity to antigen following desensitization. The blood concentration of precipitating antibody is not parallel to the anaphylactic sensitivity (COHEN and MOSKO 1943) and the active anaphylactic state may remain even though circulating antibodies can no longer be demonstrated (DOERR 1950). Thus, a quantitative study of the desensitization process *in vivo* should involve an estimation of the changes in anaphylactic reactivity of some kind of cell, known to be sensitive to antigen.

In the present paper, the desensitization phenomenon is studied by means of the anaphylactic mast-cell reaction in the nasal mucosa of the guinea pig. This method is based on a quantitative evaluation of the anaphylactic "mast-cell disappearance" from the tissue. It was found (BORÉUS 1960) that the degree of this disappearance was correlated to the dose of the antigen. It is now shown that desensitizing processes in actively anaphylactic guinea pigs can be studied with the same technique. If it is presupposed that this mast-cell response is a consequence of a reaction between administered antigen and cellular antibody, then it follows that study of the mast-cell disappearance after desensitization gives quantitative information about the antigen-antibody reaction and the anaphylactic cell reactivity in the living animal.

With the present technique it is shown that subcutaneous injections of the specific antigen inhibited the subsequent anaphylactic mast-cell response. Thus, the mast cells had been specifically desensitized, since a full anaphylactic reaction was obtained with another antigen in the experiments with double-sensitized animals. The degree of inhibition was dependent upon the amount of injected desensitizing antigen, higher doses being more effective.

It is generally considered that a desensitizing process, experimental or clinical, must proceed slowly, since a too intense supply of a desensitizing antigen will produce shock symptoms. This corresponded in these experiments to a demonstrable decrease of the mast-cell population and to the occurrence of anaphylactic symptoms after high desensitizing doses. These high doses, however, gave a very strong desensitization. Thus, high desensitizing doses gave good protection against subsequent antigen administration but were themselves accompanied by various degrees of shock symptoms, whereas small desensitizing doses gave a weaker protection but were not accompanied by any detectable anaphylactic symptoms themselves.

The state of depressed sensitivity to antigen was never absolute. Even when high desensitizing doses had been given, a mast-cell reaction could still be obtained by giving a very high challenge dose.

Desensitization was soon obtained. The mast-cell sensitivity was found to be depressed 1 hour after administration of the desensitizing dose. After about 2 weeks most of the original sensitivity had returned. It is interesting to note that the length of this period was about the same whether a large single or five smaller desensitizing doses had been given. This duration of depressed sensitivity of the mast cells is in good agreement with earlier reports, which state that the period of desensitization in the guinea pig is about 2 weeks (GAY 1935, BOYD 1956).

It is further shown that the postponement of onset of hypersensitivity is reflected in depressed mast-cell reactivity. Big doses gave a more effective inhibition of the mast-cell disappearance than did smaller ones. That desensitization of mast cells is dose-dependent is further substantiated from the experiments with prolonged administration of antigen during 5 weeks, where relatively high doses were necessary to maintain the refractoriness to antigen and where smaller doses were less effective.

The intensity of the anaphylactic shock symptoms may be evaluated also in the pentobarbital-anaesthetized guinea pig (BORÉUS 1960). It is found in this paper that the intensity of the shock symptoms corresponded to the degree of mast-cell reaction. The antigen-antibody reaction on or in different kinds of cells constitutes the anaphylactic shock symptoms. It seems that a resulting mast-cell reaction must play a major part in the genesis of anaphylaxis, since both heparin (HOLMGREN and WILANDER 1937) and histamine (RILEY and WEST 1953) are located in these cells. Furthermore, there is considerable experimental evidence to show that a smooth-muscle stimulating principle

("slow reacting substance") may be derived from mast cells in the cat (CHAKRAVARTY, HÖGBERG and UVNÄS 1959), in the rat (UVNÄS and THON 1959) and in the guinea pig (BORÉUS and CHAKRAVARTY 1960). It may, therefore, be reasonable to suppose that the occurrence of these different active principles plays an important role for the manifestation of the anaphylactic symptoms and that the inhibition of these symptoms following desensitization is mainly a result of depressed mast-cell sensitivity to antigen.

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Properties of Pacinian Corpuscles of Ulnar and Tibial Location in Cat and Fowl

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As first demonstrated by Herbst (1849), corpuscles of Pacinian type occur in great numbers between tibia and fibula in birds, where in some species strings of a length of several cm and containing hundreds of corpuscles occur, as described in detail by Schildmacher (1931). In mammals, Pacinian bodies are found at the corresponding site in the hindleg, as also in the foreleg, where they are more numerous and form conglomerates in the space between ulna and radius (Herbst 1849, Rauber 1867). Dissections of these regions in fore- and hindlegs of the cat as well as legs of pigeon and fowl, carried out for the present investigation, showed that the majority of the corpuscles are enclosed together with nerves and vessels in a sheath of connective tissue which had to be removed in order to expose the individual corpuscles and their nerve fibers.

Using conventional electrophysiological technique, recording was made from the nerve fibers of a single or a group of corpuscles. (Preliminary measurements of nerve fiber diameters of Pacinian corpuscles in cat gave an average value of 8 μ). The first series of experiments on the cat's foreleg showed that these corpuscles, like the types previously analyzed, respond to direct mechanical stimulation by a volley of impulses (Fig. 1 *A*). However, characteristic of the Pacinian corpuscles in this region is a strikingly high sensitivity to vibratory stimulation. Thus, e. g., walking on the laboratory floor was often sufficient to elicit impulse discharges, and sometimes responses could be obtained by clapping of hands or other strong auditory stimuli. When a vibratory stimulus was applied to the experimental table, discharges from Pacinian corpuscles were recorded in phase with the stimulus (Fig. 1 *B*). Both fast and slowly adapting units have been observed. The latter type may be responsible for the continuous activity observed in some units even in the absence of any intentional mechanical stimulation (Fig. 1 *C*). It is obvious that the bones to which the end organs are closely attached may transmit mechanical stimuli from various external and internal sources.

The corpuscles in the leg of the cock, which differ from those in the cat by their smaller size and more elongated shape, showed similar response patterns with a typically low threshold to vibratory stimulation. Some units reacted

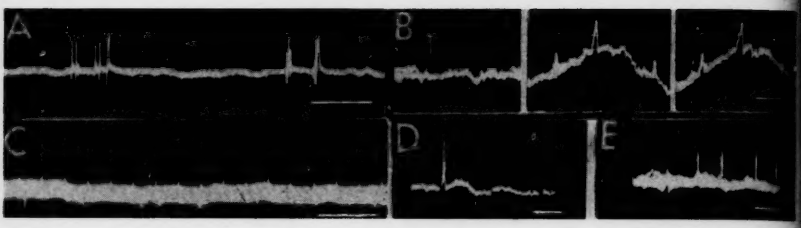

Fig. 1. Responses of Pacinian corpuscles of ulnar location in cat, *A—C*, and of tibial location in cock, *D—E*. *A*, corpuscle touched with small rod; *B*, before (straight baseline) and during vibratory stimulation at 50 per sec (curved baseline due to 50 cycles interference from vibrator); *C*, "spontaneous" discharge; *D*, single, and *E*, repetitive type of response to mechanical stimuli (synchronized with sweep) applied to corpuscles. Time bar in *A* and *C* 50 msec, in *B*, *D* and *E* 10 msec.

to mechanical stimuli by single responses (Fig. 1 *D*), while other units showed repetitive discharges (Fig. 1 *E*), which might even outlast the stimulation period.

The findings of a low discharge threshold of the encapsulated endings in a bird's leg to mechanical stimulation support the view expressed by SCHWARTZKOPFF (1949) that these sense organs mediate the high sensitivity to vibratory stimulation, which he found in training experiments also on birds rendered deaf by cochlear extirpation. Of interest in this connection from the point of view of comparative physiology are the highly differentiated sense organs in the tibia of certain insects which, as shown by AUTRUM and SCHNEIDER (1948), respond to vibratory stimuli within a wide frequency range.

Apparently the strings of Pacinian corpuscles in the cat's foreleg, although less developed than in birds, may play a role as vibratory sense organ, and it is most likely that Pacinian corpuscles of corresponding location in the cat's hindleg are the vibration receptors sought for by HUNT and MCINTYRE (1960) as mediators of the similar types of discharges recorded from the interosseous nerve of the cat's hindlimb. However, it cannot be excluded that these structures serve also other functions. Experiments on extirpation and stimulation of Herbst's organ in birds (SCHILDMACHER 1931) have indicated a proprioceptive function in muscle reflexes, and further studies of reflex effects in different species seem to be worth while.

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